THE
AMERICAN NATURALIST

Vol. LXXXVIII September-October, 1954 No. 842

THE THEORY OF ADAPTIVE POLYMORPHISM

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Dobzhansky (especially 1951) has proposed the theory that polymorphism as such is frequently adaptive. He states (1951, p. 123) that if of two inversions coexisting in the same population one is found to be favored by selection in summer and the other in winter, then populations with both inversions will be at an advantage to populations with only one, because these will be at a disadvantage either in summer or in winter according to which inversion is present. He suggests that polymorphism will enable a species to exploit the environment more efficiently. Consequently, widespread species occurring in many habitats should be more polymorphic than those with restricted ranges. He produces evidence that this is so by comparing several species of Drosophila.

While this interesting theory may be correct, it seems that much more experimental evidence is required to support it, for the following reasons:

(1) It is not easy to understand what is meant by one population being at an advantage or being more highly adapted to a particular environment, compared with another, and no sufficiently detailed definition has yet been made. Dobzhansky has stated (1949, p. 132; 1951, p. 123) that when there is balanced polymorphism, with heterozygotes at an advantage, in a population, the "average adaptive value" ($\bar{w}$) of the population is at a maximum when equilibrium is reached. Consequently selection acts in such a way as to produce the maximum adaptive value in the population. This wording certainly suggests that a population in genetic equilibrium with heterozygotes at an advantage is in some way highly adapted. But it appears from his definition of adaptive value (1951, p. 116) that what is meant by this expression is selective value. The apparent use of "adaptation" as a synonym for "selection" does not seem to be in accordance with general practice (Darwin, 1859, and autobiography in F. Darwin, 1888; Fisher, 1930; Sommerhof, 1950). Adaptation (that is, fitness for a particular function in a particular environment) is a much wider concept than selection and can in many instances be measured by methods which involve no reference to selection coefficients. Moreover, the selection coefficient for any one gene expresses (in terms of progeny) the net effect of all the different se-

lection pressures acting on the various effects of that gene. Consequently it can be at best only a poor estimate of the degree of adaptation produced by any one of these effects. In *Primula vulgaris*, Crosby (1949) has shown that a "gene" can spread because of an immediate intraspecific advantage (efficiency of pollination of its own and other mating types) which nevertheless produces not only a decrease in viability but also an inevitable destruction (at least in part) of an outbreeding mechanism. Clearly the selection coefficient for this gene is not a good estimate of the adaptation allowing pollen grains from plants carrying it to achieve successful fertilization in competition with other pollen grains (if this can be called an adaptation) since it takes account also of the reduced viability. Moreover, it gives no indication of the breakdown of the outbreeding mechanism which could well involve the extinction of the population. The spread by selection of such unfavorable gene-complexes has been treated in general terms by Fisher (1941).

When "adaptive value" is replaced in Dobzhansky's definitions by selective value, it is seen that his conclusion means that selection will alter the gene (or inversion) frequencies in such a way that there will be a stable equilibrium if the heterozygotes are at an advantage compared with the homozygotes, or fixation of that gene (or inversion) the homozygote of which has an advantage over the other genotypes. This is, of course, correct, but tells us nothing about the adaptation of the population concerned.

Indeed, until a precise definition of adaptation and advantage as referring to populations has been made, it will be impossible to devise experiments or collect data which will confirm or refute the hypothesis unambiguously. The consequences of different definitions are very diverse. For example (to take only two of the possible definitions), if those populations more likely to persist through environmental changes are considered to be the better adapted, then we should investigate the degree of variability in general (not only of polymorphism) and determine which populations are more likely to produce suitable genotypes when conditions change. But two populations may be exploiting (in some sense) their present environments identically in every way, and yet one may be more likely to survive than the other if it has a greater reserve of genetical variation. Consequently the present ecological state of each population cannot be taken as an indication of probable persistence. On the other hand, those populations which maintain a greater density per unit area under the same environmental conditions may be considered better adapted. If there is variation in density from population to population under the same conditions, then obviously the individuals of the different populations must be reacting differently to these conditions. This fact, of course, tells us almost nothing about the different probabilities of persistence of different populations. On this definition it would be necessary to show that the existence of polymorphism in some populations is actually the cause of their having a high density, which can happen only if the genes controlling the polymorphism are counteracting negatively density dependent factors (Haldane, 1953).

The behavior of genotypes competing in the same population gives no direct evidence on the ability of separate populations each homozygous for one of the alternative genotypes, to survive in the wild. A particular allelomorph may even be inviable when homozygous in the presence of an alternative allelomorph, as for example if more rapidly growing larvae eat so much more food that the slower growers are unable to get enough to bring them to pupation. Yet the disadvantageous homozygote (producing slow growth) may be perfectly viable as a pure stock in the wild; in fact, for equal numbers of eggs, more of the slow-growing stock may reach maturity than of a stock of rapid growers. On the other hand, a particular homozygote may be at an extreme disadvantage because of extensive dislocations of its structure and physiology that result from homozygosity, irrespective of what genotypes may be coexisting with it in the same population. In this situation the selection coefficient relative to other genotypes does give a rough measure of the homozygote's chances of survival in pure cultures. But a mere inspection of selective values of different genotypes in the same population does not allow one to distinguish between these two types of situation. Consequently, if in the presence of AR chromosomes in *Drosophila pseudoobscura*, ST chromosomes are at a selective advantage during the hot part of the year, and at a disadvantage during the cool part, this cannot be taken to mean that therefore a population containing only ST chromosomes will be in any sense at a disadvantage to others with both during the cold season. It is a matter for further experimental investigation to determine whether they are or not.

(2) If the suggestion (Dobzhansky, 1951, p. 110) that polymorphism within a single breeding community increases "the efficiency of the exploitation of the resources of the environment by the living matter" is correct, then in some sense polymorphic populations could be considered as better adapted than monomorphic ones. But this suggestion rests on an extension of Gause's principle to genotypes within the same population, an extension which needs careful examination. The analogy between species and genotypes certainly appears unsatisfactory in systems involving balanced polymorphism with heterozygotes at an advantage, in which several genotypes of different selective values do in fact continue to coexist in the same population and environment, although they appear on Dobzhansky's hypothesis to correspond to species as closely related as possible, which according to Gause should be unable to coexist. Moreover, the situation is unlike any contemplated by Gause, since any one of the "species" can appear amongst the progeny of the other two.

If on the other hand, genes (or inversions) are considered as corresponding to species, then in balanced polymorphism the allelomorphs concerned have the same selective value when in equilibrium, whereas the whole point of Gause's principle is that two species will not have or will not maintain the same efficiency in the same environment and will not arrive at a state of stable equilibrium. If it be claimed that an allelomorph in a homozygote is in a different environment from the same allelomorph in a heterozygote,

then the proposed extension of Gause's principle may perhaps be acceptable. Even so, it still remains to be shown that there is in fact any increase in efficiency of exploitation of the external environment by reason of the polymorphism. Dobzhansky remarks (1951, p. 110), "A single genotype, no matter how versatile, could hardly function with maximal efficiency in all environments. Hence, natural selection has preserved a variety of genotypes, more or less specialized to render the organism efficient in a certain range of the existing environments." But this is to assume the conclusion. Is it true that the different genotypes in stably polymorphic populations are exploiting the external environment in even slightly different ways? This again is a matter for ecological research not for *a priori* genetical deduction.

(3) Dobzhansky and his colleagues have shown in some species of Drosophila that common and wide-ranging species tend to possess both a greater diversity of chromosomal inversions and a higher frequency of them in each population investigated, than closely related species which are more restricted in range and less abundant. These interesting and important facts have been interpreted as evidence that "the more polymorphic a species is, the more environments it can use or control" (Dobzhansky, Burla and da Cunha, 1950; see also Dobzhansky, 1951, p. 133). This could mean either (a) that a more polymorphic species can extend its range into a greater number of regions which differ considerably ecologically, or (b) that it can utilize a greater range of materials and conditions at any one place or, of course, both.

The greater polymorphism of the more widely ranging forms could however be the result, not the cause, of the range inhabited. In breeding communities in different environments different inversions are likely to be favored when they arise, and therefore a greater degree of polymorphism may be produced in wide-ranging species than in geographically restricted ones. In the most widespread and abundant species the chances that a generally advantageous inversion will arise are greatest; such inversions may then spread through the range of the species, increasing the number of kinds of inversion at any one locality. Consequently, the facts elucidated by Dobzhansky need not necessarily be regarded as supporting his hypothesis. Furthermore, it is known that some wide-ranging and common species have almost no inversions in wild populations, so that comparisons between different species, which may have very different capacities for variation in this respect, should be treated with reserve, as da Cunha *et al.* (1953) have pointed out.

The alternative hypothesis, that a polymorphic species can exploit (in some sense) a greater range of materials or conditions at any one locality, is also not necessarily supported by Dobzhansky's evidence, which consists only of information on distribution and frequencies of particular inversions. Polymorphism may be maintained by the superior viability or fertility for example of the heterozygotes, quite independent of heterogeneities in the immediate environment.

Allison (1954) has shown that in regions where malaria caused by *Plasmodium falciparum* is common, the gene producing sickle-cell is also com-

mon. The reason is that in these areas, while the homozygote (si si) is highly disadvantageous because it produces sickle-cell anemia, and the normal homozygote (Si Si) is liable to severe attacks of malaria, the heterozygote is comparatively free from these disadvantages. Obviously, if the sickle-cell homozygote were not so disadvantageous, the gene would merely spread throughout these areas exactly as genes producing industrial melanic forms have done in Lepidoptera (Ford, 1953). The polymorphism is a result not of heterogeneities in the environment within malaria-infested regions but of the relative effect of the sickle-cell gene upon the individual, when homozygous and when heterozygous.

Da Cunha (1951) found that the relative selective values of the homo- and heterozygotes for some inversions in *Drosophila pseudoobscura* vary greatly according to the species of yeast or bacteria given as food. But in several cases, the polymorphism was maintained by selective superiority of the heterozygotes even when only one food-species was present. Moreover, it appears that all the food-species were eaten by all the chromosomal types. There seems no reason to believe that a pure stock of one chromosomal type could not maintain itself successfully on any or all of the food-species, but da Cunha's experiments were not designed to investigate this point. Consequently there is no evidence either way as to whether the effect of the chromosomal polymorphism in this example is to increase "the efficiency of the exploitation of the resources of the environment."

Dobzhansky and his colleagues have also shown that, in some species of Drosophila at least, polymorphism decreases towards the edges of the range, and is greatest where the greatest diversity of foods may be expected to occur (da Cunha, Burla and Dobzhansky, 1950). This may mean only that towards the edges of the range selection is more stringent and fewer inversions are able to maintain themselves. But in any case, no evidence that selection is affecting the degree of polymorphism in different populations is really relevant for the discussion of the hypothesis. It is necessary in all cases to show whether the presence of polymorphism is really affecting the adaptedness of the populations concerned (in whichever sense this may be defined in future).

SUMMARY

The hypothesis of adaptive polymorphism as proposed by Dobzhansky cannot as yet be considered well established. It is generally agreed that genetical variability enables a species to evolve in response to changes. Dobzhansky has made the important suggestion that variability also allows a population to exploit a constant, or a changing, environment more efficiently than a single genotype could do. Consequently he believes that arrangements which maintain variability, notably balanced polymorphism, give the populations in which they occur an adaptive superiority over others.

However, it appears that by adaptive polymorphism is usually meant only polymorphism maintained by selection. Such polymorphism can occur in a population without affecting its adaptation. The relative adaptive

value of populations is a very difficult concept which has not been clearly defined. Observations on relative coefficients of selection of different polymorphs within a population give no direct evidence on the ability of any one polymorph to survive as a pure stock. And further, the evidence which has been adduced to support the hypothesis is capable of different interpretations. In no case has direct evidence been obtained that different polymorphs in a population are in any sense exploiting the environment in different ways, and thereby affecting the adaptedness of the population significantly.

ACKNOWLEDGEMENTS

We are very grateful to Dr. E. B. Ford, F.R.S., for detailed criticism of the manuscript, and to Professor Th. Dobzhansky, Professor Sir Ronald Fisher, F.R.S., and Professor J. B. S. Haldane, F.R.S., for valuable comments. One of us (P. M. Sheppard) is indebted to the Nuffield Foundation for their generous support.

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Vol. LXXXVIII, No. 842 The American Naturalist September–October, 1954

ACROPETAL AUXIN TRANSPORT AND XYLEM REGENERATION—A QUANTITATIVE STUDY*

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The strongly polar movement in plant tissues of the growth hormones known as auxins is a fact of great importance for the growth and differentiation of the plant. Some examples of the many processes apparently conditioned by the polar movement of auxin are the inhibition of axillary buds during "apical dominance" (Thimann and Skoog, 1934), the strictly basipetal movement of the phototropic curvature in Avena coleoptiles (Went and Thimann, 1937), the formation of roots on the basal end of many stem cuttings (Went and Thimann, 1937), the growth pattern of bean hypocotyls (Jacobs, 1950a and 1950b), and the basipetal regeneration of xylem cells around a wound severing a vascular strand in Coleus stems (Jacobs, 1952).

That substances showing such manifold effects should be the only hormones known to us which have such strictly controlled polar movement within plant tissues makes that polarity of great interest.

In this paper, new evidence and new calculations will be presented to show that the hypothesis that "auxin at physiological concentrations moves only basipetally in shoots" does *not* hold true for all plants, although well justified for those species and shoot organs which so far have been critically and thoroughly investigated.

The strict basipetal polarity of auxin transport in sections from the Avena coleoptile was demonstrated by Went and White (1939), using an elegant new technique which allowed the sections to be followed individually. They both emphasized and demonstrated the crucial importance of preventing movement of applied auxin in the surface film of water which readily forms on sections placed in high humidities (such as are routinely used in the Avena bioassay for auxin). Unless such surface movement is prevented, claims that "acropetal auxin movement has occurred" are obviously worthless. Surface drying of sections and lowering of the relative humidity to 80 per cent or less are usually sufficient to remove all traces of acropetal auxin transport as long as physiological concentrations of auxin are used (e.g., ±2 p.p.m. as in Jacobs, 1950a, 1951; Went and White, 1939). Went and White demonstrated that some acropetal transport did occur through coleoptile sections when such high auxin concentrations as 1000 p.p.m. were used; however, there was no reason to consider it of any significance for the normal plant.

In addition to Avena coleoptiles, strictly basipetal movement of auxin has been demonstrated in sections from the hypocotyls of various species (Dijkman, 1934, on Lupinus; Jacobs, 1950a, on Phaseolus; van Overbeek,

*Report of research supported in part by a grant from the American Cancer Society and in part by funds of the Higgins Trust allocated to Princeton University.

1933, on Raphanus), the stems of tomato and Tagetes (Skoog, 1938, and Went, 1941, respectively), and the gynophore of the peanut plant (Jacobs, 1951, on *Arachis hypogaea*). Since surface-film artifacts would, of course, show no polarity with respect to the direction of auxin movement, the fact that the researchers cited above found no trace of auxin in their *apical* collecting blocks demonstrates that they were dealing with the movement of auxin through (rather than on) the sections. This conclusion is valid even though very critical precautions against surface-film artifacts were not always taken.

Unfortunately, most of the papers presenting reports of auxin movement in both acropetal and basipetal directions do not satisfy the stricter requirements for critical work under these conditions. From those papers reporting acropetal movement from direct tests, the results of most must be considered equivocal because strict precautions were not taken against surface-film artifacts (Mai, 1934; Jost and Reiss, 1936; Höfner, 1937; Oserkowsky, 1942; Nystérakis, 1947). Some of these authors also applied unphysiologically high concentrations of auxin (Höfner and Nystérakis). Others increased the probability of getting physiologically meaningless results by running their transports on excised sections for very long periods (Mai, for 20 hours; Jost and Reiss, overnight; Oserkowsky, up to 24 hours; Nystérakis, for 12 hours). The authors of a recent paper, investigating quantitative differences in acropetal auxin-transport in flowering stems as contrasted to vegetative ones, have also apparently not routinely used special precautions against surface-leakage (Leopold and Guernsey, 1953). In addition, they have substituted for the standard Avena auxin-bioassay a newly devised root-growth assay for which neither specificity nor reproducibility are demonstrated or claimed. And their table 1 shows half as much "equivalent μg IAA" from one set of sections as from the duplicate set (the only set in the paper ringed with vaseline). Furthermore, the size of this reported difference between "identical" treatments is more than enough to account for all the differences between corresponding levels of flowering and vegetative stems which they show in their figure 2.

In contrast to this list, there are only two reports of *acropetal* auxin transport which meet the requirements of technique mentioned above. One is Went's interesting paper on inverted Tagetes cuttings (1941), the other is a paper on xylem regeneration in Coleus internodes (Jacobs, 1952). Because of the importance of auxin in the control of plant growth and differentiation, these two reports of acropetal transport are worth particularly careful scrutiny. Went's report concerns 2-noded stem cuttings which had been induced to root at the originally distal end by the local application of high concentrations of auxin. These "apically" rooted cuttings were then planted, rooted end down, and grown in this inverted position. A control group of cuttings was rooted at the originally basal end and subsequently grown in the normal orientation with respect to gravity. Axillary buds near the non-rooted end were allowed to grow out as the cuttings became established.. At various intervals thereafter, sections were cut from the internodes

and transport tests were run. The tests were essentially standard in technique; no corrections for diffusible auxin were apparently made. Results are shown in figure 1. Went interpreted these results as showing that

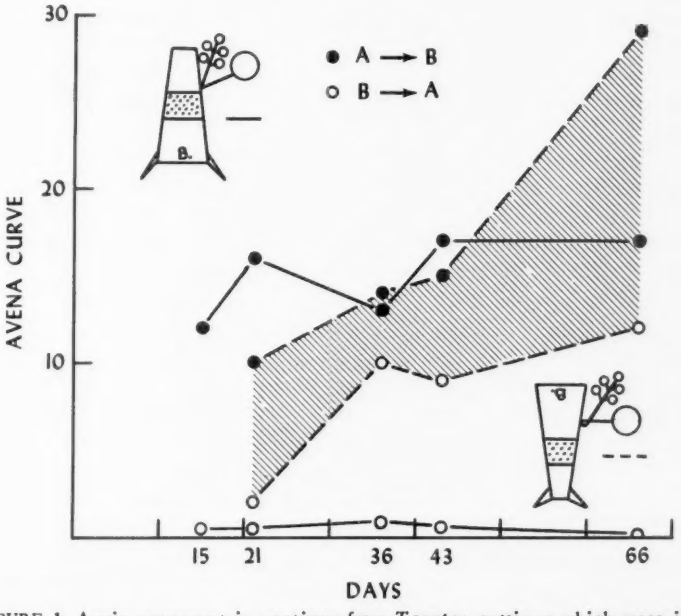

FIGURE 1. Auxin movement in sections from *Tagetes* cuttings which were in the normal and the inverted orientation with respect to gravity. The stippled regions on the diagrams indicate the general location of the sections used for transport tests. "A → B" designates movement in the *original*-apex toward *original*-base direction, and similarly for "B → A." Data from Went (1941).

"polarity as such is not reversed, but that auxin transport in the original direction continues at about the same rate and that a second auxin transport in the opposite direction is added." The occurrence of acropetal auxin-movement in the inverted cuttings seems indisputable. Although there is no mention of special precautions against surface-leakage, the fact that sections from *normal* cuttings showed such strict basipetal movement makes it extremely unlikely that the acropetal movement in inverted cuttings was due to surface movement. Whether the auxin movement in the original-apex to original-base (i.e., basipetal) direction "continues at about the same rate" or is steadily increasing is not clear, in my opinion; unfortunately, the data are available only as average Avena curvatures (instead of as "indole-acetic acid equivalents") so transport values on different days are not exactly comparable. The appearance of this acropetal movement is apparently not connected with mere inversion with respect to gravity, since transport tests on sections from the original base (now zenithward above the elongating side-branch) showed strictly basipetal auxin-movement. It

was, then, only sections intercalated between the growing side-shoot and the root system which developed the new transport.

The results of this intriguing experiment immediately suggest the question, "Is the basipetal transport, found in sections isolated from cuttings grown under these inverted conditions, of significance in the physiology of the plant?" There is, of course, no reason to think that it even occurs in the normal stem-cutting (figure 1). And it has not been investigated further to determine if it is of significance for the physiology of the inverted cutting.

It *is* important, I believe, for students of auxin transport to provide evidence that their results, obtained with small excised sections, capped on each end with agar blocks and placed in dark humid chambers, apply to the normal, intact plant. Quantitative evidence of this sort exists for strictly basipetal auxin movement in coleoptiles. The phototropic curvature of the coleoptile, related on the basis of many experiments to auxin redistribution, moves strictly basipetally at a rate of 16mm./hour (Went and Thimann, 1937, p. 178), while the rate of basipetal auxin movement in excised sections is 9.6 mm./hour (Went and White, 1939), a reasonably close agreement.

The second paper containing critically run experiments which demonstrated acropetal auxin movement, was one concerned primarily with internal factors controlling the regeneration of xylem in Coleus stems (Jacobs, 1952). It was found that "the strong basipetal movement of auxin is paralleled by a strong basipetal polarity of xylem regeneration; the relatively small acropetal movement of auxin is paralleled by a slight acropetal differentiation of xylem." The movement of auxin was determined in standard transport tests, using sections from intact normal plants, the regeneration of xylem by counting the number of strands of xylem-cells which regenerated in plants normal and intact except for a single severed vascular strand in internode No. 2. Because this work on Coleus represents the only report of acropetal auxin movement in normal plants at normal concentrations of auxin with qualitative evidence as to its significance in the normal physiology of the plant, further research has been done in an attempt to investigate this phenomenon more closely and to see if quantitative relations could be found.

MATERIALS AND METHODS

In most details, materials and methods are the same as used in the earlier research (Jacobs, 1952). Variability of the experimental material was drastically reduced, as before, by using a clonal stock of Coleus, rigorously selecting plants on the basis of stem-height, leaf-lengths, strength of leaf-pigmentation, growth-rates, and vegetative condition (i.e., omitting flowering plants). The region of the plant investigated was carefully defined, also: "Internode No. 2" was used throughout (figure 2). It is the internode immediately below leaf-pair No. 2 (Leaf-pair No. 1 is the pair of leaves which, on the day of selection, have most recently completed their unfolding from the apical bud). From a somewhat wider range of leaf

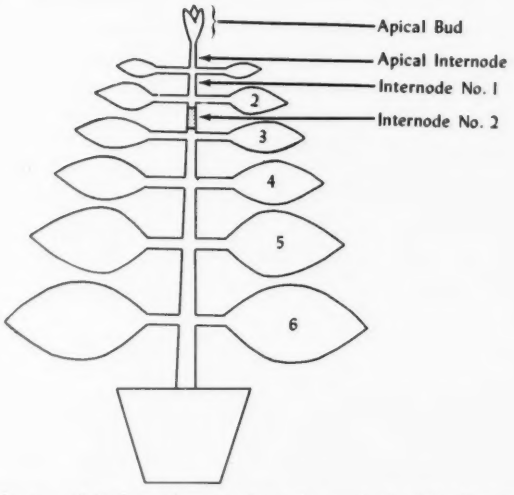

FIGURE 2. Diagram of *Coleus* plant to show the method of designating leaves and internodes. Internode No. 2 (stippled) was the internode used in these studies on xylem regeneration and auxin transport. (The leaves, which are actually decussate, are here represented as being all in one plane.)

lengths, only those plants were used which had lengths of 70–100 mm. for leaf-pair No. 2.

An important addition was made in the method of running transport tests. In addition to the precautions against surface-leakage which were used in the 1952 paper (i.e., lowered humidity in the transport chamber, thorough surface-drying of the sections, and omitting to add water drops to the cut surfaces), a complete ring of vaseline was tightly pressed around each individual transport-section.

As before, the possibility of unconscious bias was ruled out by making measurements of Avena curvatures and counts of xylem strands from coded material. The treatment represented by any given shadowgraph or cleared internode was consequently not known while measurements or counts were being made.

Bioassay for auxin was done as before, using the standard Avena test, except that an additional "calibration" concentration of 0.005 p.p.m. IAA was used to supplement the concentration of 0.025 p.p.m. IAA.

AMOUNT OF ACROPETAL TRANSPORT

Because of the likelihood of getting surface-leakage as an artifact in transport tests, it was felt to be the responsibility of any investigator finding auxin in the "acropetal" collecting block of agar to rule out this factor before interpreting his results as evidence for acropetal movement *through* the sections. The earlier experiments on Coleus (Jacobs, 1952) were carried out using the precautions cited above. They had been found to be sufficient

for other material by Went and White (1939) and the writer (Jacobs, 1950a, 1951). However, to make assurance doubly sure, a test was run with vaseline rings as waterproof barriers to surface-leakage. The average Avena curvatures for the various tests were converted to equivalent IAA concentrations by using the curvatures obtained from the "calibration" blocks of IAA on each day. That is, by expressing the results as the amount of IAA which would give the same amount of curvature, a correction can be made for the varying sensitivity of the Avena bioassay from day to day and, more important, the results from different days can be validly compared. Results of all replications using the simpler precautions of the 1952 paper are presented in table 1, along with the results of the experiment using vaseline rings.

TABLE 1

AMOUNTS OF AUXIN, EXPRESSED AS "mg. IAA $\times 10^{-6}$," COLLECTED IN COMBINED TRANSPORT + DIFFUSION TESTS ON SECTIONS FROM NO. 2 INTERNODES OF *COLEUS*

Test No.	No vaseline rings						Vaseline rings	Over-all average
	4	6	8	9	10	Av.		
1) Acropetal	19	....	15	27	16	19	26	20.3
2) Basipetal	92	72	37	52	52	61	87	65.3
Ratio $= \frac{2)}{1)}$	4.8	....	2.5	1.9	3.2	3.2	3.3	3.2

It is immediately clear that the more rigorous experiment agrees exactly with the earlier ones so far as concerns the relative amounts of auxin transported acropetally and basipetally. The ratio of basipetal to acropetal movement is 3:1 in No. 2 internodes. Statistical tests show, in addition, that the absolute amounts of auxin collected at apex and base in the "ringed" experiment are not significantly different from the average amounts collected in the non-ringed experiments.

TRANSPORT AND XYLEM DIFFERENTIATION

Now that we know more exactly the amounts of auxin which can move acropetally and basipetally in No. 2 internodes, we are in a position to reinterpret earlier experiments. In the 1952 paper, it was found that when one of the main, longitudinally running vascular strands in internode No. 2 was severed by a V-shaped cut and leaf-pairs 2 and 1 and the apical bud (i.e., the major auxin-sources distal to this internode) were excised, there resulted a marked and significant decrease in the amount of xylem-regeneration relative to the leaf-on controls. But regeneration did not drop to zero. Not being cognizant of the actual extent of acropetal auxin movement (as we are now from the experiments reported above), I attributed this "residual" xylem regeneration to auxin normally present in the internode (p. 307). However, now that we know that one-third as much auxin can move *up*

the internode as can move down, we would expect that in such an experiment all the proximal auxin-sources (leaf-pairs 3–6) would be supplying *acropetally* moving auxin to the regenerating area. The data summarized in table 2 provide evidence that this is the case. By comparing the ratio

TABLE 2

RELATION OF PROXIMAL AND DISTAL AUXIN-SOURCES TO THE NUMBER OF XYLEM STRANDS REGENERATED IN THE NO. 2 INTERNODE OF *COLEUS*

	Average no. of strands regenerated		Observed ratio
	*Calculated	Observed	
When only proximal auxin-sources are present	5.0	5.6	1.0
When distal auxin-sources are present		16.0	2.9

*Calculated on the assumption that xylem regeneration is directly proportional to the amount of acropetal and basipetal auxin transport as shown on the right-hand side of Table 1 $\left(\text{i.e., by solving the proportion: } \frac{20.3}{65.3} = \frac{\times}{16.0}\right)$.

of auxin-transport in opposite directions (table 1) with the ratio of the amounts of xylem-regeneration obtained when auxin is available from opposite directions, we see that there is an astonishingly exact parallel. When there are only proximal auxin-sources available, xylem regeneration drops to a level which accurately reflects the amount of auxin which the regenerating internode can transport acropetally. When distal sources are available, too (or when *only* distal sources are available—p. 305 of the 1952 paper), the amount of xylem formed is proportional to the greater basipetal auxin-transport.

These results, therefore, provide evidence that the primary limiting factor for xylem regeneration in these internodes is not the amount of auxin which the leaves are producing, but rather the *transport capacity* of the internode. (This conclusion is also supported by indications, from the absolute amounts of auxin diffusing out of proximal leaves, that these leaves are producing more auxin than the internode can transport.)

DISCUSSION

The occurrence of a substantial amount of acropetal auxin movement has been demonstrated in No. 2 internodes of vegetative Coleus plants. One third as much auxin moves acropetally as moves basipetally. The significance of this unusual movement in the normal physiology of the plant has been verified by experiments which show an exact, quantitative relationship between amounts of auxin transported (or added distally) and the amounts of xylem regenerated.

These results demonstrate that the *strict* basipetal polarity of auxin movement which has been found and thoroughly investigated in coleoptiles and hypocotyls does *not* occur in all Angiosperm shoot-structures.

How general the occurrence of acropetal auxin movement is we do not, of course, know. Only a minute sample of species and of well-defined developmental stages have been thoroughly investigated so far. But there are a few indications that acropetal movement of auxin occurs in other places than in young internodes of Coleus. Kaupp (1937) obtained Avena curves of 5.3° *vs.* 2.1° for basipetal *vs.* acropetal auxin diffusion in sections from Anemone stems; but he ignored the 2.1° in making an interpretation. Acropetal auxin was also ignored by van der Laan (1934), whose data show 21 + ° *vs.* 4.8° ± 0.6 for basipetal *vs.* acropetal diffusion from young stems of *Vicia Faba.* My results with young stem sections of Coleus suggest that Kaupp and van der Laan may have actually been observing acropetal auxin-movement without being aware of it.

At any rate, the assumption that auxin movement can be counted on to be strictly basipetal no matter what the stem-part being studied is obviously no longer justified. And those results which have, in the past, been explained in terms of an assumed absence of acropetal auxin movement should be re-examined.

Having shown how different these young Coleus internodes are from coleoptiles and hypocotyls, one might next wonder whether they differ from older internodes on the same plant. We would certainly expect so from earlier work on other organs, since graded changes in the amounts of auxin transported have been observed in both coleoptiles and hypocotyls (Went and White, 1939; and Jacobs, 1950, respectively), both organs whose anatomical characteristics undergo much less drastic changes along their axes than do Coleus stems. The expectation was fulfilled in a recent transport test using vaseline-rings to prevent surface-leakage. Both absolute amounts of auxin and relative amounts moving acro- and basi-petally were different in the older internodes, which had substantial cambial activity.

There are a number of points of interest in connection with the demonstration of a quantitative relation between the amount of auxin transport and the amount of xylem regeneration in a stem. First, it all adds still more weight to the extensive evidence, presented earlier (Jacobs, 1952), that the differentiation of xylem cells in a regenerating vascular strand is normally controlled by auxin produced by the leaves. A summary of the total evidence is:

(A) Xylem regeneration is predominantly basipetal in internode 2 of the "intact" plant, but some acropetal differentiation occurs.

(B) The movement of both applied auxin and natural auxin ("diffusible auxin") is predominantly basipetal through these same internodes.

(C) The young leaves (particularly leaf No. 2) are the main sources of free-moving auxin; but there are only small amounts of auxin present in internode No. 2.

(D) When physiological concentrations of auxin are added to sections cut from No. 2 internodes, three times as much auxin is transported basipetally as is transported acropetally.

(E) Excising all the leaves *below* the regenerating internode has no effect on the amount of xylem-regeneration, so long as the *distal* leaves are still on.

(F) Excising all the leaves *above* the regenerating internode (i.e., leaves 1, 2 and the apical bud—the major auxin sources on the shoot) markedly reduces the amount of xylem-regeneration. The number of xylem-strands differentiated under these conditions exactly reflects the *acropetal* transport capacity of the No. 2 internode; that is, xylem regeneration is now being limited directly by auxin produced by the leaves *below* No. 2 internode but is limited indirectly by the amount of that proximally supplied auxin which No. 2 internode can transport acropetally.

The second point of interest is that, although the evidence is clear that auxin is the normal limiting factor for xylem regeneration in these Coleus internodes, yet the *indirect* limiting factor is the transport-capacity of the internode for auxin. In other words, the factor which controls how much auxin gets to the regenerating area is *not* how much auxin is produced by the leaves but, unexpectedly, how much auxin can be transported by the stem. This fact is emphasized here because now we know that it is not an isolated phenomenon: in studying the control of growth in bean hypocotyls by auxin moving basipetally, evidence was found that the increasing transport-capacity of the hypocotyl-tip was acting as an indirect limiting factor on the growth of the subjacent region (Jacobs, 1950a, 1950b).

To researchers interested in the mechanisms by which the growth and differentiation of vascular plants are coordinated and controlled, it is intriguing that transport-*capacity* should be found to be the indirect limiting factor in the only two cases where such a relation has been thoroughly investigated. The paucity of information quantitatively relating the *production* of auxin to various auxin-controlled processes may, partially at least, result from the more general occurrence of transport-capacity rather than auxin-production as the limiting factor.

A third point worth calling attention to is concerned with the value of "diffusible" auxin *vs.* "extractable" auxin. Many papers during the last 20 years have been concerned with various methods of extracting plants for material active in the Avena bioassay. Solvents, pH, temperature, enzyme-treatments, duration of extraction, etc., have been widely varied with consequent wide variation in the amount of auxin-activity obtained. Although there is an inherent interest in finding out just how much auxin-activity can be derived from a given wet-weight of plant material, to a developmentalist it is regrettable that greater "auxin-yield" has been pursued to the almost complete exclusion of concern with *physiologically significant* auxin-content. One of the very few papers in which the significance of extractable *vs.* diffusible auxin has been investigated is that of Went on growth and tropisms in decapitated Avena coleoptiles (1942). Almost all cases in which there have been demonstrations of a close, quantitative relation between auxin and physiological processes have been cases where

diffusible auxin was being measured. Some examples are: normal inhibition of leaf-abscission (Wetmore and Jacobs, 1953); photo- and geotropisms (Went and Thimann, 1937; and Went, 1942); control of growth in bean hypocotyls (Jacobs, 1950 a, b); control of xylem differentiation in Coleus (Jacobs, 1952, and this paper). I do not mean to imply that extractable auxin is *a priori* physiologically meaningless. (In fact, some extraction methods give auxin activities more or less identical with those obtained by diffusion.) However, the time is long overdue for specific evidence relating the differing amounts of extractable auxin to processes going on in the plant.

SUMMARY

Earlier experiments on the polarity of auxin-transport in shoots are critically discussed. The following points are emphasized: The importance of short-time experiments; the value of using physiological auxin-concentrations; the need for relating transport data to normal physiological processes; and, in particular, the necessity of guaranteeing that no movement of auxin is occurring in a surface film of water.

Previous research on the relation between auxin and xylem regeneration in vegetative Coleus shoots has been extended and quantified. Evidence is presented that:

1. In young No. 2 internodes the ratio of basipetal to acropetal auxin-transport is 3 : 1, when physiological auxin concentrations are used.

2. The amount of xylem regenerated when a vascular strand is severed is limited by the amount of auxin which can reach the regenerating area from the leaves.

3. The amount of auxin which can reach the regenerating area is, in turn, normally limited by the transport-capacity of the internode.

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Vol. LXXXVIII, No. 842 The American Naturalist September-October, 1954

CRYPTIC SUBSPECIATION IN DROSOPHILA BELONGING TO THE SUBGENUS SOPHOPHORA[1]

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INTRODUCTION

It is generally believed that subspecies often represent incipient species and that, therefore, knowledge of many details of the speciation process might be expected to be revealed by studies of subspecies in genetically well-known organisms—especially subspecies of the sort designated "borderline" by Patterson and Dobzhansky (1945) and characterized by development of almost, but not quite, enough reproductive isolation to be considered good species.

The genetically best-known species belong to the genus Drosophila. Yet, among the 274 Drosophila named in Patterson and Stone's (1952) recent classic on evolution in this genus, there are only 15 forms that fall in their seven groups of subspecies, all belonging to the subgenus Drosophila.

In the subgenus Sophophora there is the *willistoni* group which includes the very interesting *willistoni* sibling group that has been described in detail by Dobzhansky (1946) and Burla *et al.* (1949). *D. tropicalis* Burla and da Cunha belongs to this sibling group. In general the centers of geographic distribution of these sibling species are in Brazil.

Collections of the *willistoni* sibling species taken by the writer, first in Cuba and later throughout the Greater Antilles, included a previously undescribed subspecies of *D. tropicalis*. The present paper describes this subspecies, which is named *D. tropicalis cubana*, and compares it morphologically and genetically with the South American subspecies which, following taxonomic convention, is now designated *D. tropicalis tropicalis*.

FORMAL DESCRIPTION OF NEW SUBSPECIES

Drosophila tropicalis cubana, subspecies nova

♂. Arista with 10–13 branches. Antennae yellow, third segment somewhat darker and with rather long dense pilosity. Front over $\frac{1}{3}$ width of head, wider above; dull yellow. Anterior orbital about $\frac{9}{10}$ and middle about $\frac{2}{3}$ posterior. Two or three prominent oral bristles, second nearly as long as first. Carina short, gradually falling off below; rounded; neither sulcate nor nose-like. Face and cheeks pale yellow; latter's greatest width about $\frac{1}{8}$ greatest diameter of eyes. Eyes bright red with short light colored pile.

[1] Publication No. 12, Journal Series of the Atkins Garden and Laboratory of Harvard University, Cienfuegos, Cuba. Contribution No. 101 from the Department of Zoology and Entomology, The University of Tennessee. Supported in part by a grant from the Penrose Fund of the American Philosophical Society.

Achrosticals in 6 rows; no prescutellars. Anterior scutellars parallel. Mesonotum and scutellum tannish yellow, slightly shining. Pleurae and legs pale yellow; apical bristles on first and second tibiae, preapicals on all three. Sternoindex about 0.33.

Abdomen yellow, slightly shining; each segment with an uninterrupted diffuse dark brown apical band fading out laterally.

Wings clear. Costal index about 1.9; fourth vein index about 1.9; 5x index about 1.7; 4c index about 1.3. Dorsal bristle at apex of first costal section stouter, but not longer, than ventral one. Third costal section with heavy bristles on basal ½.

Length body 2.14 mm. (living specimen); wing 1.95 mm.

♀. Length body 2.46 mm.; wing 2.22 mm.

Internal characters of imagines.

Testes pale yellow, with about 3 inner and 2 outer coils or gyres; vasa deferentia not fused, or distal ends partially fused for distance up to four times diameter of single vas deferens.

Spermathecae subspherical, width being somewhat greater than height, chitinized, dark brown; ventral receptacle a flat spiral with about 7 coils, bent into W-shaped plate lying against vagina.

Two anterior and two posterior Malpighian tubes, ends free.

Other characteristics, relationship, and distribution.

Eggs.—Two filaments, apical ⅔ much expanded, thin, spatula-like; about ⅔ length of egg.

Puparium.—Yellowish tan. Each spiracle with about 9–11 branches on very short stalk.

Chromosomes.—Metaphase plate with one pair of rods and two pairs of V's.

Relationship.—Belongs to the willistoni group of the subgenus Sophophora.

Distribution.—Collected in the Greater Antilles: Soledad Central, Cienfuegos, Cuba; Baracoa, Cuba; Constant Spring, Jamaica; Port-au-Prince, Haiti; Ciudad Trujillo, Dominican Republic; Río Piedras, Puerto Rico; and Caribbean National Forest, Puerto Rico.

Holotype male.—Holotype and paratypes descendants of a single female collected at Soledad Central, September 1950; holotype and some paratypes to be deposited in American Museum of Natural History; some paratypes deposited in collection of Department of Zoology and Entomology, University of Tennessee, Knoxville.

Note.—Females of *D. tropicalis tropicalis* and *D. tropicalis cubana*, on the one hand, differ from those of the other sibling species in that the latter have about five more coils in their ventral receptacles and also have less chitinized spermathecae.

MATERIALS

At the outset of this study nine strains of *D. tropicalis cubana* from Soledad Central, Cienfuegos, Cuba, a strain of *D. equinoxialis* from Tefé, Amazonas, Brazil, and one strain each of *D. tropicalis tropicalis, D. willistoni*, and *D. paulistorum* from Belém, Pará, Brazil, were available. Later strains of *D. tropicalis cubana* from Baracoa Cuba, Constant Spring, Jamaica, Port-au-Prince, Haiti, Ciudad Trujillo, Dominican Republic, and Río Piedras, Puerto Rico, and, most recently, a strain of *D. tropicalis tropicalis* from Içana, Amazonas, Brazil, became available.

MORPHOLOGICAL DIFFERENCES

Since no morphological differences were readily discerned between the Belém *tropicalis* and Cienfuegos *cubana*, a careful comparison of some of their morphological traits was undertaken. For this purpose each subspecies was grown in an uncrowded culture under optimal feeding condi-

tions at 24°–26° C. Soon after hatching the flies were transferred to shell vials containing fresh food and left there to harden for two days. Next, measurements to determine body length, wing length, costal index, 4th vein index, and 5th vein index were made on 46–50 females and 50 males of *tropicalis* and 5–6 females and 5–6 males from each of 9 strains of *cubana*. Following the convention of Burla *et al.* (1949), body length was recorded as the sum of the distances from the antenna to the base of a haltere and from the latter to the end of the anal tubercle. The number of branches of the arista was determined for females only, using 50 specimens of *tropicalis* and 7 from each of 7 strains of *cubana*.

Table 1 summarizes the data for these traits. It appears that *cubana* is a somewhat larger fly—at least the *cubana* female is larger than the *tropicalis* female, even though there is no statistically significant difference between the males of the two species—and has a slightly longer wing. The costal index is also greater in *cubana*. But the 5x index is significantly greater in *tropicalis*. This last difference may not be the property of all strains of the respective subspecies. There is probably no difference between the two subspecies with respect to 4th vein index (significantly

TABLE 1

COMPARISON OF SOME MORPHOLOGICAL TRAITS IN *D. tropicalis tropicalis* BURLA AND DA CUNHA AND *D. tropicalis cubana* SUBSP. N.*

Trait	Subspecies	N	Range	M ± m	Student's *t*	P
Body length (mm.)	*tropicalis* ♀	50	2.14–2.65	2.38 ± 0.016	3.17	< 0.003
	cubana ♀	54	2.20–2.75	2.46 ± 0.017		
	tropicalis ♂	50	1.88–2.38	2.12 ± 0.016	1.50	> 0.13
	cubana ♂	54	1.92–2.38	2.14 ± 0.011		
Wing length (mm.)	*tropicalis* ♀	50	1.96–2.22	2.11 ± 0.007	8.54	≪ 0.0001
	cubana ♀	54	2.00–2.35	2.22 ± 0.011		
	tropicalis ♂	50	1.73–2.06	1.90 ± 0.01	3.86	< 0.0002
	cubana ♂	54	1.76–2.06	1.95 ± 0.009		
Costal index	*tropicalis* ♀	46	1.74–2.14	1.90 ± 0.011	6.61	≪ 0.0001
	cubana ♀	54	1.77–2.28	2.03 ± 0.015		
	tropicalis ♂	50	1.64–1.89	1.76 ± 0.03	6.69	≪ 0.0001
	cubana ♂	53	1.55–2.07	1.87 ± 0.013		
4th vein index	*tropicalis* ♀	46	1.77–2.13	1.95 ± 0.013	2.00	0.04–0.05
	cubana ♀	53	1.76–2.23	1.99 ± 0.013		
	tropicalis ♂	50	1.74–2.19	1.94 ± 0.014	0.45	0.60–0.70
	cubana ♂	53	1.64–2.22	1.93 ± 0.017		
5x index	*tropicalis* ♀	46	1.50–1.99	1.68 ± 0.015	3.04	< 0.002
	cubana ♀	54	1.38–1.96	1.61 ± 0.017		
	tropicalis ♂	50	1.48–1.97	1.71 ± 0.047	0.43	0.60–0.70
	cubana ♂	53	1.46–1.97	1.70 ± 0.016		
Branches of arista	*tropicalis* ♀	50	10–12	11.12 ± 0.07	1.82	0.70–0.80
	cubana ♀	49	10–12	10.90 ± 0.10		

*N = number of flies; M = mean; m = standard error; P = probability.

different between females at the 5 per cent level but not at the 1 per cent level) or with respect to number of branches of the arista.

Although some means differ significantly, the range of values for all traits studied overlap broadly. The absolute differences between corresponding means are small, being in several instances differences in the second decimal place. It is apparent that these traits could not be used with any reliability to separate individuals belonging to the two subspecies, regardless of whether or not the flies developed under optimum conditions. No morphological differences other than those just described have yet been discovered between the subspecies when either these same strains of the subspecies or the other Greater Antilles strains of *cubana* and the Içana strain of *tropicalis* were examined.

CHROMOSOMES

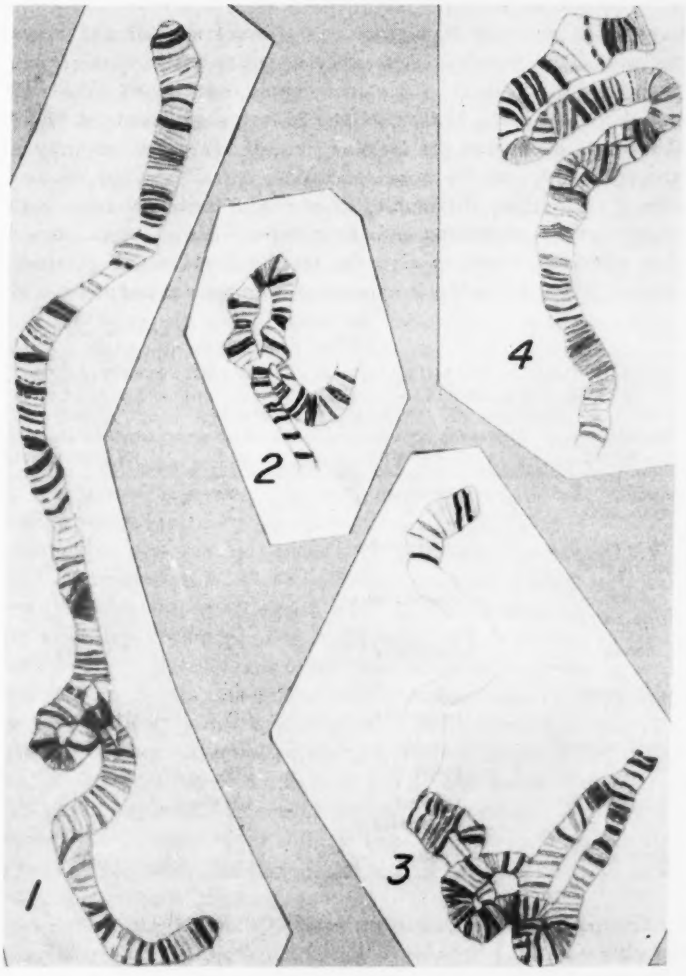
The metaphase plate figures in the ganglion cells of both *tropicalis* and *cubana* appear identical. The gene arrangements in the two subspecies may be compared by examining the larval salivary gland chromosomes of F_1 hybrids; inversion loops in the chromosomes of these hybrids may indicate the presence of any distinctive arrangements. Seven strains of *cubana* from Cienfuegos, five from Ciudad Trujillo, two from Río Piedras and one each from Baracoa, Constant Spring, and Port-au-Prince were intercrossed with Belém *tropicalis*. At least one *cubana* strain from each of the localities was used to obtain hybrids by both reciprocal intercrosses with the *tropicalis*. This Belém strain of *tropicalis* was homozygous for a single gene arrangement in each of its five detectable chromosome arms.

Pairing was often complete in all the chromosomes of the hybrids. In every hybrid at least two inversion loops were observed: a medium-long one in the proximal third of chromosome II L and a small inversion in the middle third of chromosome III. The former inversion is depicted singly in figures 1 and 2 and with a tandem, more basal inversion in figure 3. The inversion in chromosome III is depicted in figure 4.

Only four inversions, two in III and two in II L, have previously been reported in *tropicalis* (Burla *et al.*, 1949; Dobzhansky, Burla, and da Cunha, 1950). The inversions found in *tropicalis-cubana* hybrids do not correspond with any inversions already known.

The subbasal inversion in II L has been found heterozygous in *cubana* from wild populations of Haiti, Dominican Republic, and Puerto Rico. In fact this inversion is the only one yet discovered heterozygous in natural populations of *cubana*. It has also been observed to form a loop in Belém *tropicalis*—Jamaica *cubana* F_1 hybrids.

The fact that no heterozygous inversion was observed in the progeny of more than 50 *cubana* females taken at Soledad Central, in conjunction with the lack of heterozygotes for the II L subbasal inversion in hybrids between *cubana* from this region and Belém *tropicalis*, suggests that the *cubana* of Cuba may be, in the subbasal region of II L, homozygous for the same gene arrangement as is the Belém *tropicalis*.



FIGURES 1–4. Heterozygous inversions in hybrids between *Drosophila tropicalis tropicalis* and *Drosophila tropicalis cubana*. Figs. 1–2. A medium-long inversion in the left arm of the second chromosome. The entire chromosome arm is depicted in fig. 1. Fig. 3. Same inversion in tandem with a subbasal inversion. (An unclear region of the chromosome is represented as bandless.) Fig. 4. Distal two-thirds of the third chromosome showing a small inversion in the distal third of the chromosome.

In any event, the differences between gene arrangements in the two subspecies are relatively slight.

SEXUAL ISOLATION

In order to determine the insemination relations possible within the *willistoni* sibling group, Burla *et al.* (1949) made a number of "no choice"

experiments in which 10 females of one of these species and 10 males of another were aged 4–6 days separately and then without etherization confined together at about 25° C. in food-containing 25 × 95 mm. shell vials (some experiments using 20 females and 20 males were made in 35 × 95 mm. vials) for 8–12 days; then the females were dissected and their spermathecae and ventral receptacles examined microscopically for spermatozoa.

Table 2 summarizes the results of a similar series of tests between a Cienfuegos strain of *cubana* and the other sibling species and, for comparative purposes, includes also the results Burla *et al.* obtained using *tropicalis*. From this table it appears that *tropicalis* and *cubana* differ in

TABLE 2

NUMBERS OF FEMALES DISSECTED (n) AND PERCENTAGES FOUND INSEMINATED IN SOME EXPERIMENTS INVOLVING NO CHOICE OF MATES (DATA MARKED WITH AN ASTERISK (*) FROM BURLA *et al.*, 1949)

Males	Females	n	% inseminated
cubana	*willistoni*	36	33.3
tropicalis	"	112	0.0*
cubana	*paulistorum*	217	14.3
tropicalis	"	115	27.8*
cubana	*equinoxialis*	142	0.0
tropicalis	"	143	0.0*
cubana	*tropicalis*	63	90.5
willistoni	*cubana*	81	0.0
"	*tropicalis*	105	3.8*
paulistorum	*cubana*	229	0.0
"	*tropicalis*	104	65.0*
equinoxialis	*cubana*	183	0.0
"	*tropicalis*	105	0.0*
tropicalis	*cubana*	122	68.0

that males of the latter can inseminate *willistoni* females with relatively high frequency while *tropicalis* males are completely sexually isolated from these females, and that *tropicalis* females frequently may be inseminated by *paulistorum* males and occasionally by *willistoni* males while sexual isolation between these males and *cubana* females is complete.

In order to determine whether these results obtained with a single strain of *cubana* might be expected with other geographical strains, the "no choice" experiments were repeated on a small scale using one strain of *cubana* from each of the countries in which this subspecies has been collected. For any particular cross not more than 20 females were recovered and dissected. It was found that some strains differed from each other in their insemination relations with the different sibling species. Although insemination frequencies differed somewhat (probably not significantly in such small samples) from strain to strain, in general the males of all these

cubana strains paralleled those of the Cienfuegos strain in possible inseminations. The females of some strains of *cubana* were inseminated by *equinoxialis* males, a relationship shown neither by the Cienfuegos strain nor by *tropicalis*; otherwise, the insemination relations of *cubana* females were about the same as those of the Cienfuegos females.

Male "multiple choice" experiments were made to determine if any sexual isolation exists between *tropicalis* and *cubana*. The Belém strain of *tropicalis* and *cubana* strain CC-4 from Cienfuegos were used. Virgin females and males were collected and aged separately for 4–6 days; then 10 females of each subspecies—marked by clipping a slight nick in either the right or left wing, depending upon the subspecies—and 10 males of one subspecies were transferred without etherization to 25 × 95 mm. shell vials containing food and left there at 25° C for one hour, the average time required for about 50 per cent of the females to be inseminated if 10 females and 10 males of *tropicalis* are so confined together. At the expiration of the time limit, the contents of the vials were etherized, the females of the two subspecies separated, and the spermathecae and ventral receptacles examined microscopically for spermatozoa.

Data obtained in the "multiple choice" experiments are summarized in table 3. In the experiments *tropicalis* males inseminated significantly more of their own females than of *cubana* ($\chi^2 = 25.32$; $P \ll 0.0001$), while *cubana* males were about equally successful in inseminating either kind of female ($\chi^2 = 0.12$; $P > 0.90$). The Levene (1949) coefficient of isolation for *tropicalis* males is 0.33 ± .06, and 0.02 ± .07 for *cubana* males. The former coefficient is significantly different from zero, but the latter is not. The Levene joint coefficient of isolation, which measures the reproductive isolation between the two subspecies, is 0.18 ± .05. The latter is significant ($t = 3.95$). It thus appears that sexual isolation may exist to a slight extent between the two subspecies due to some sexual isolation between *tropicalis* males and *cubana* females—but there is none between *tropicalis* females and *cubana* males.

When both kinds of "multiple choice" experiments are considered, it becomes apparent that a significantly greater proportion of *tropicalis* females than of *cubana* females (69.4 and 57.1 per cent respectively) were inseminated ($\chi^2 = 11.88$; $P < 0.001$). The Levene coefficient of excess

TABLE 3

NUMBER OF FEMALES DISSECTED (n) AND PER CENT INSEMINATED (%) DURING EXPERIMENTS IN WHICH MALES OF ONE SUBSPECIES HAD A CHOICE BETWEEN FEMALES OF BOTH SUBSPECIES

♂♂	*tropicalis* ♀♀		*cubana* ♀♀		χ^2	Levene coefficient of isolation
	n	%	n	%		
tropicalis	191	73.8	189	48.7	25.32	0.33 ± .06
cubana	169	64.5	172	66.3	0.12	0.02 ± .07

insemination of *tropicalis* over *cubana* is 0.15 ± .05. This means that gene flow is somewhat greater from *cubana* into *tropicalis* than in the reverse direction, under conditions of the experiments.

The results of these "multiple choice" experiments may very well be due to greater sexual activity on the part of *tropicalis* females than of *cubana* females. As will be shown in the next section, this hypothesis is also supported by some evidence other than the fact that more *tropicalis* females than *cubana* females were inseminated in these experiments.

FERTILITY AND FECUNDITY

At 25° C. reciprocal intercrosses were made between Belêm *tropicalis* and seven strains of *cubana* from Cienfuegos, four from Baracoa, two from the Dominican Republic, two from Puerto Rico, and one from Haiti. Five or more flies of each subspecies were used in a cross. Every cross produced progeny; but, when the F_1 flies were serially transferred to fresh food, in no case was an F_2 obtained. An intercross of Cienfuegos *cubana* and Içana *tropicalis* gave similar results.

In the same fashion as just described, five *cubana* strains, one from each country in which this subspecies has been collected, were reciprocally intercrossed with each other and with Belêm *tropicalis*. All possible intercrosses (a total of 30) between these strains were made. Every cross produced an F_1. While an F_2 was easily obtained from each of the intercrosses between geographical strains of *cubana*, no F_2 could be obtained from any intercross of *tropicalis* and *cubana*. The F_1 flies from each of the 30 crosses were separated by sex, and a backcross was then attempted between each sex and each parental strain (four backcrosses for each original intercross). The F_1 males and females from intercrosses of geographical strains of *cubana* were fertile with either parental strain. On the other hand, the F_1 males from all intercrosses involving *tropicalis* were sterile no matter whether the backcross was attempted with *tropicalis* or *cubana*, while the sisters of these sterile males were fully fertile with males of either subspecies.

In order to compare the frequencies with which *tropicalis-cubana* intercrosses and backcrosses successfully produce offspring and also the relative numbers of resulting progeny, "crossability" experiments of the type made by Patterson and Dobzhansky (1945) were performed using Belêm *tropicalis* and *cubana* strain CC-4 from Cienfuegos. In each of the experiments 140–170 single pairs of virgin females and males, previously aged separately for 3–5 days, were confined with food in individual creamers at 25° C; on the fifth day of confinement any creamer in which either fly had died was discarded; on the twelfth day the proportion of remaining creamers in which progeny were evident was recorded. Of each of the different types of crosses attempted, the first 50 creamers recorded as having housed a fertile pair were tagged in order that the emerging progeny could later be counted as a measure of fecundity. (In all, seven such creamers subsequently had to be discarded for technical reasons.)

The data on fertility and fecundity as measured in these experiments are summarized in table 4. In this table *tropicalis* is symbolized by T and *cubana* by C, with the female parent being given first. F_1 hybrids are represented TC or CT according to whether the female parent was *tropicalis* or *cubana*, respectively.

The fertility data in table 4 show that once again all intercrosses between *tropicalis* and *cubana* produced fertile hybrid females and completely sterile hybrid males. It should also be noted that the *tropicalis* control crosses were more successful (82.6 ± 3.9 per cent fertile) than the *cubana*

TABLE 4

NUMBER OF PAIR MATINGS (n) SCORED AND PER CENT FERTILE, ALSO NUMBER OF CULTURES IN WHICH THE OFFSPRING WERE COUNTED (N) AND MEAN NUMBER OF OFFSPRING PER FERTILE MATING (M). *Drosophila tropicalis tropicalis* ABBREVIATED T; *Drosophila tropicalis cubana*, C. FEMALE PARENT SHOWN FIRST.

	Matings	N	% fertile	n	M ± m
Controls	T × T	92	82.6 ± 3.9	50	44.0 ± 3.4
	C × C	79	63.3 ± 5.3	44	44.9 ± 4.7
P_1	T × C	103	71.8 ± 4.4	50	49.4 ± 3.9
	C × T	101	70.3 ± 4.5	50	53.1 ± 3.7
Backcrosses	TC × T	129	72.9 ± 3.9	50	81.2 ± 3.4
	CT × T	110	80.9 ± 3.7	49	74.2 ± 3.5
	TC × C	88	88.6 ± 3.4	50	80.0 ± 3.9
	CT × C	120	91.7 ± 2.5	50	78.4 ± 3.5
	T × TC	82	sterile		
	T × CT	138	"		
	C × TC	104	"		
	C × CT	138	"		

ones (63.3 ± 5.3 per cent fertile). The frequencies of fertile intercrosses were intermediate (70.3 per cent and 71.8 per cent) between the comparable control frequencies. For the backcrosses of hybrid females the range varied from a frequency (72.9 per cent) about the same as for the intercrosses to frequencies (88.6 per cent and 91.7 per cent) in excess of that for the *tropicalis* controls.

In all successful kinds of crosses involving *tropicalis* males the frequencies of fertile matings (70.3–82.6 per cent) with the four different kinds of females did not differ significantly ($\chi^2 = 6.18$; $P > 0.10$). This may be interpreted to mean that the different kinds of females were on the average equal in fertility. However, when the different kinds of crosses involving *cubana* males are compared, it is seen that the frequencies of fertile matings (63.3–91.7 per cent) did differ significantly ($\chi^2 = 32.38$; $P \ll 0.0001$). If this was not due to differences in fertility of the females, then it appears likely that the success with which *cubana* males mate is very much dependent upon the genotype of the females—hybrid females, as well as *tropicalis* females, probably being more active sexually than are *cubana*

females. If this is true, mating success of *tropicalis* males is less dependent upon the relative level of sexual activity of the females than that of the males themselves when an excess of time (more than 5 days) is allowed for mating.

The absence of progeny from crosses attempted with hybrid males was not due to failure of these males to copulate. On the contrary, hybrid males mated rather readily with parental type or hybrid females, but transferred no spermatozoa. In fact, hybrid males do not produce any motile spermatozoa. Microscopic examination of the testes of hybrid males disclosed bundles of degenerating spermatids throughout most of the gland and only degeneration masses in the proximal region. Meiosis was not studied.

The mean numbers of offspring produced in the control crosses and different kinds of *tropicalis-cubana* intercrosses and backcrosses are presented in the last column of table 4. The means for the controls and intercrosses were about equal (44.0–53.1), as were those for backcrosses of hybrid females (74.2–81.2); however, the latter were significantly greater than the former. This means that the hybrid females were heterotic—the heterosis being expressed as increased fecundity.

DISCUSSION

Of the seven sets of Drosophila subspecies listed by Patterson and Stone (1952) *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* in the nature of their relationships (Patterson and Dobzhansky, 1945) most closely resemble *D. tropicalis tropicalis* and *D. tropicalis cubana*. In both cases the subspecies are easily intercrossed to produce viable hybrids; the F_1 male hybrids are completely sterile and have degenerating spermatids in their testes; sexual isolation, if any, between the subspecies is slight and confined to one of the reciprocal intercrosses; and there is relatively little chromosomal reorganization. Two distinctive inversions have developed between *tropicalis* and *cubana*, but only one long inversion between *pallidipennis* and *centralis*. The extent of chromosomal reorganization between the latter two subspecies remains therefore the least yet known between two such forms.

Although female hybrids between *pallidipennis* and *centralis* are generally somewhat less fertile than females of either subspecies, the fertility of female hybrids between *tropicalis* and *cubana* does not differ significantly from that of either parental type of female. Furthermore, the increased fecundity of *tropicalis-cubana* female hybrids shows them to be heterotic. Heterotic subspecific hybrids are not unique; Wharton (1944) found that hybrids between *D. mercatorum mercatorum* and *D. mercatorum pararepleta* exceeded their parents in vigor and fertility. Since *tropicalis-cubana* hybrid females are no less fertile than females of either subspecies, the increased fecundity of the hybrids would offset to a considerable extent the partial block that male hybrid sterility presents to gene exchange should these subspecies meet and hybridize in nature. Nonetheless, steril-

ity of the hybrid males is the only well-developed reproductive isolating mechanism possessed by these subspecies.

The northern-most distribution reported for *tropicalis* is in the Territory of Rio Branco, Brazil (Burla *et al.*, 1949); the southern-most distribution now known for *cubana* is in Jamaica. Although collecting in the Lesser Antilles would be expected to extend the distribution of one or both of these forms, it appears certain that the extent of reproductive isolation between them is insufficient to permit maintenance of distinctive sympatric populations; therefore, divergence to the level of full species has not yet occurred. Nevertheless, the differences already described, when taken in conjunction with geographic replacement, are quite adequate for ranking the forms as distinct subspecies.

The ranking of *tropicalis* and *cubana* as subspecies is independent of the fact that these forms are only known to differ morphologically in the mean measurements of certain characters that overlap to such an extent as to be completely useless for classifying individuals; in contrast to this, Patterson and Dobzhansky (1945) were rather easily able to separate *pallidipennis* and *centralis* grown under uniform conditions, and also managed to separate about fifty per cent of the individuals that had not been grown under uniform conditions. Morphological differences between *tropicalis* and *cubana* are even more cryptic than those found between the other sibling subspecies: *americana* and *texana* (Patterson, Stone, and Griffen, 1940); *meridiana* and *rioensis* (Patterson, 1943); *mercatorum* and *pararepleta* (Wharton, 1944); and *fulvimacula* and *flavorepleta* (Patterson, 1952).

Notice should be taken of the fact that *tropicalis* and *cubana* belong to the *willistoni* sibling species group which includes three species other than these subspecies. The morphological differences between the sibling species are so slight that it is very nearly impossible to separate on such a basis the females of three species. Spassky and Frota-Pessoa (personal communication) are able to classify the males of some strains of all four species by means of rather subtle differences between the externally visible genitalia. Still, the greatest morphological differences known between the four species are between the ventral receptacles and extent of chitinization of the spermathecae of the *D. tropicalis* subspecies and those of the other three species. While *tropicalis* and *cubana* have diverged genetically, they have not abandoned the morphological conservatism characteristic of the sibling group to which they belong.

Although morphologically *tropicalis* and *cubana* have diverged only to a nearly imperceptible extent, they show considerable differences in general level of adaptedness as reflected by their frequencies relative to the other siblings sympatric with them. Dobzhansky and Pavan (1950) have shown that in Brazil the *willistoni* sibling group is dominant in most collections of Drosophila. Da Cunha *et al.* (1950) found that usually the most widespread of the sibling species, *D. willistoni*, is also the most abundant. According to personal communication from Dobzhansky and da Cunha the only locality in which they have found *tropicalis* to be dominant is in

Marajó Island, an island previously reported by da Cunha *et al.* to be ecologically unfavorable for the *willistoni* siblings. Ordinarily *tropicalis* was outnumbered about 50 to 1 by the other siblings in the Brazilian collections. In the Greater Antilles, the *willistoni* siblings made up about 9 per cent to about 36 per cent of the total samples (from Cienfuegos and Port-au-Prince, respectively) but were only second to fourth in abundance. The best data on relative frequencies of the different siblings are available for Cienfuegos and Ciudad Trujillo. In the former locality *cubana* outnumbered *willistoni* almost 2 to 1 and was about equally as frequent in the latter. As much as a third of the *willistoni* sibling sample (in Río Piedras) was *D. equinoxialis*. These relations make it apparent that *cubana* is better adapted to compete with the species most closely related to it than is *tropicalis*.

ACKNOWLEDGEMENTS

The writer is deeply indebted to Professor Th. Dobzhansky and Dr. Howard Levene for many helpful criticisms and suggestions; to Mr. and Mrs. Boris Spassky for the many kindnesses they extended during much of the work; and to Mr. Frederic F. Primich and Mrs. E. Alston for technical assistance. It is also a pleasure to express gratitude to the following for their exceptional hospitality and assistance during the collecting: Dr. and Mrs. I. D. Clement, Atkins Garden and Laboratory, Cienfuegos; Srs. Miguel and Pablo Arrúe, Baracoa; Mr. and Mrs. R. P. Bengry and Mr. G. R. Proctor, Science Museum, Jamaica Institute, Kingston; Mr. and Mrs. W. C. Cumming, Institute of Inter-American Affairs, Ciudad Trujillo; Dr. George N. Wolcott, Agricultural Experiment Station, Río Piedras, and Dr. Honorico Ciordia, formerly of that station; and Mr. Frank H. Wadsworth, U. S. Forestry Service, Caribbean Division, Río Piedras.

SUMMARY

A new subspecies, *Drosophila tropicalis cubana*, belonging to the siblings of the *willistoni* group of the subgenus Sophophora and distributed throughout the Greater Antilles is described and compared with the South American subspecies, *Drosophila tropicalis tropicalis* Burla and da Cunha. The two subspecies overlap widely in the ranges of values for certain morphological traits conventionally used in describing species of Drosophila; although some of the means differ significantly, not one of the traits can be used for separating the subspecies with any degree of reliability. Therefore, the morphological differences are cryptic. Sexual isolation between the subspecies is minor, if it exists at all, and confined to one of the reciprocal intercrosses, both of which produce viable hybrids—fertile females that are heterotic as shown by increased fecundity and completely sterile males that have testes containing degenerating spermatids. At present the subspecies are best distinguished by intercrossing with known strains and examining the fertility of the F_1 hybrids. The gene arrangements of the subspecies differ only slightly, by a medium-long inversion in II L and a very small one in III. The northern subspecies is generally

better adapted to compete with the closely related siblings than is the South American subspecies. There are some differences between the sexual isolation of each of the subspecies from the other siblings, but these may be strain rather than subspecific differences.

Subspeciation of *D. tropicalis* is compared with that of other Drosophila, especially with *D. pallidipennis* subspeciation which it most nearly resembles.

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Vol. LXXXVIII, No. 842 The American Naturalist September–October, 1954

THE INTRINSIC RATE OF NATURAL INCREASE IN A POND SNAIL (*PHYSA GYRINA* SAY)

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The intrinsic rate of natural increase (rate of increase per head per day) has been calculated for several species of insects and mammals. The results obtained were formulated from mean data of fairly large numbers of individuals. These papers have been reviewed by Evans and Smith (1952).

This parameter (r) has not been investigated from the viewpoint of the individual and it is obvious that, although this measure of population growth is genetically determined and exhibits a species constancy, a variation within the species can be anticipated. In view of this, it appears to be worthwhile to present these data based upon observations made on the pond snail, *Physa gyrina*. In addition, it was possible to observe the effects of density upon the value of r and to compare the values of r for laboratory specimens reared under the same conditions from stock obtained in two separate localities.

METHOD

During this study, an attempt was made to keep the physical environment (aeration, temperature, light, food) for all snails reared as nearly constant as possible. The laboratory stock of *P. gyrina* was obtained from a small backwater pond of the Huron River at Scio, Michigan, and a series of small rocky pools in communication with the Huron River at the base of Argo Dam within the city limits of Ann Arbor.

Snails to be reared were selected at time of hatching and kept in constantly aerated water in glass containers in isolation and in groups of two, six and eight individuals. Their daily life history was accurately documented. After they had begun to oviposit, each egg mass was removed from the parent's container, placed in a separate dish and observed daily until hatching. The data gathered in this manner included (1) the age, in days, of the snails at the time oviposition began, (2) the numbers of eggs laid on succeeding days, (3) the number of eggs hatching in each mass and (4) the number of embryos dying and the stage in which they died. A complete life table for each individual or group of individuals is available.

For convenience symbols are used to designate the snails reared individually and in groups from the Scio and Argo habitats (table 1).

The method employed by previous authors (Birch, 1948; Leslie and Park, 1949; Evans and Smith, 1952) in determining the value of the intrinsic rate of natural increase (r) was to use trial values for r in satisfying the equa-

TABLE 1

THE PLAN OF EXPERIMENTS SHOWING THE NUMBER OF SNAILS USED FROM THE TWO LOCALITIES SCIO AND ARGO

SCIO	
S1-1–S1-6	6 snails reared in isolation
S1s	Mean data of S1-1 through S1-6
S2*	2 snails reared in two liters of water
S6a	6 snails reared in two liters of water
S6b	6 snails reared in one liter of water
*all snails except S2 came from the same egg mass	
ARGO	
A1-1–A1-3	3 snails reared in isolation
A1s	Mean data of A1-1 through A1-3
A8	8 snails reared in two liters of water
all from the same egg mass	

tion $\Sigma e^{-rx} l_x m_x = 1$. The symbol l_x is the probability of a female being alive at time x (days after birth), m_x is the number of female eggs laid at time x which hatch and presumably attain maturity. In this study, all mortality occurred in the egg stages. Hence l_x has been taken as unity and mortality shifted to the m_x curve. *P. gyrina* is monoecious so the necessity of establishing sex was eliminated.

Much of the laborious procedure involved in calculating r was eliminated in this work by a graphic solution. At least two trial values for r were used for each set of data. The first trial value was obtained by the formulation $T = \frac{\log_e R_o}{r}$ where R_o is the net reproductive rate and is equal to the sum

TABLE 2

SCIO SNAILS—EGGS LAID AND HATCHED PER SNAIL

Days after birth	S1-1	S1-2	S1-3	S1-4	S1-5	S1-6	S1s	S2	S6a	S6b
77–86										33.67
87–96									11.00	31.84
97–106				222		34	42.66	39.50	17.17	11.66
107–116	98			86	29	99	50.99	87.50	32.34	11.49
117–126	271	17		204	2	102	99.33	51.00	48.17	50.16
127–136	422	44	98	113		219	149.49	17.50	37.83	65.00
137–146	73	53	50			9	30.82	11.50	8.33	6.00
147–156	10	17	5				5.33	26.50	3.00	4.84
157–166	1	20				37	9.68	30.00	4.17	7.17
167–176	123			59		10	32.00	1.50	1.33	8.34
177–186	58			55		3	19.33		1.17	7.67
187–196	21		46	46		8	21.84			6.66
197–206	5		34	37		27	17.18			2.17
207–216			67	2		53	20.34			1.50
217–226	5		9	1		22	6.17			
227–236	37						6.17			
237–246	24						4.00			

TABLE 3

ARGO SNAILS—EGGS LAID AND HATCHED PER SNAIL

	A1-1	A1-2	A1-3	A1s	A8
207-216	259	....	....	86.33	....
217-226	113	97	10	73.33	....
227-236	166	11	111	95.00	....
237-246	51	....	16	22.33	3.750
247-256	200	384	199	261.00	10.250
257-266	150	41	29	73.33	13.750
267-276	36	....	2	12.67	0.625
277-286	8	....	....	2.67	1.000
287-296	....	....	....	....	6.125
297-306	2	....	....	0.67	0.875
307-316	13	....	5	6.01	1.000
317-326	9	....	19	9.33	0.125

of $l_x m_x$ (in this case all of the eggs laid by a snail which hatched). T is the mean generation time and was tentatively taken to be the time in days when half of the eggs ($R_o/2$) had been laid. The second trial value was chosen by inspection so that the summation was not less than 0.1000 nor greater than 10.0000. The summations were plotted on the abscissa and the corresponding r value on the ordinate of semi-logarithmic paper. All points plotted for a single set of data fell on, or near, a straight line. The value of r corresponding to the value of unity on the abscissa was read off from these graphs. These are the values of r shown in table 5.

To check the accuracy of extrapolating values of r when the summations fell only on one side of unity, another trial r was chosen so that points fell

TABLE 4

THE PROPORTION OF THE TOTAL LIFE SPAN SPENT IN THE REPRODUCTIVE, PRE- AND POST-REPRODUCTIVE PERIODS

	Period development (%)	Period reproduction (%)	Period post-reproduction (%)	Total life span (days)
S1-1	26	28	46	448
S1-2	24	14	62	509
S1-3	27	19	54	485
S1-4	15	20	65	634
S1-5	24	3	73	479
S1-6	20	23	57	524
S1s	22	18	60	514
S2	23	16	61	440
S6a	21	24	55	414
S6b	23	38	39	346
A1-1	50	28	22	412
A1-2	53	12	35	409
A1-3	51	25	24	443
A1s	51	22	27	422
A8	46	14	40	528

TABLE 5

THE INTRINSIC RATE OF INCREASE AND RELATED STATISTICS FOR SNAILS IN ALL EXPERIMENTS

	Mortality of eggs (%)	Number eggs laid per snail	r	T	R_0	Number days to double population
S1-1	32	1701	0.0543	130	1148	12.77
S1-2	52	315	0.0362	139	151	19.15
S1-3	44	549	0.0380	151	309	18.24
S1-4	31	1201	0.0576	117	825	12.03
S1-5	26	42	0.0307	112	31	22.58
S1-6	52	1290	0.0510	126	623	13.59
S1s	40	850	0.0494	126	513	14.03
S2	43	450	0.0475	118	265	14.59
S6a	33	245	0.0439	116	165	15.79
S6b	9	272	0.0522	106	248	13.28
A1-1	45	1839	0.0300	231	1007	23.14
A1-2	7	573	0.0259	242	533	26.76
A1-3	55	867	0.0241	248	391	28.76
A1s	41	1093	0.0273	237	643	25.39
A8	21	46	0.0138	264	38	50.22

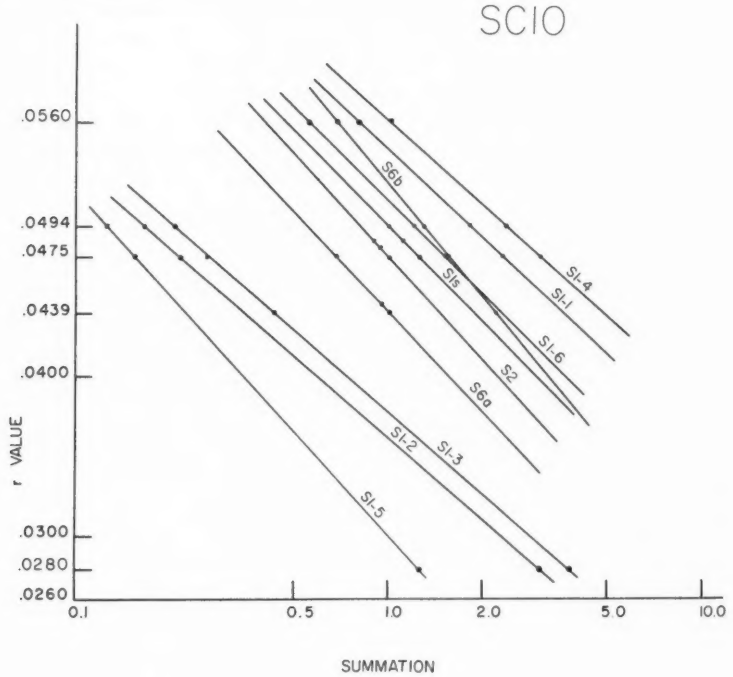

FIGURE 1. Graph showing the summation $\Sigma e^{-rx} l_x m_x$ for trial values of r for snails from Scio.

on both sides of unity. This was done for S1-1 through S1-6 and S6b (values were computed for S1s, S2 and S6a). A value for r was determined for S1-2, S1-3 and S1-5 without a summation for r equal to 0.0280 (fig. 1) and also with a plot for that value. Considering the latter procedure to give a closer approximation, S1-3 and S1-5 were lowered 0.0002 and 0.0003 respectively (the value for S1-2 remained the same). The maximum error was 0.98 per cent. The same procedure was carried out for S1-1, S1-4, S1-6 and S6b; first without a summation for r equal to 0.0560 and then with that value included (fig. 1). Only one of the previously extrapolated values was changed, S1-4 was raised 0.0001—an increase of 0.17 per cent. These errors are comparatively small and since the r value is an approximation, it appears to be reasonably accurate to extrapolate values of this parameter from two points.

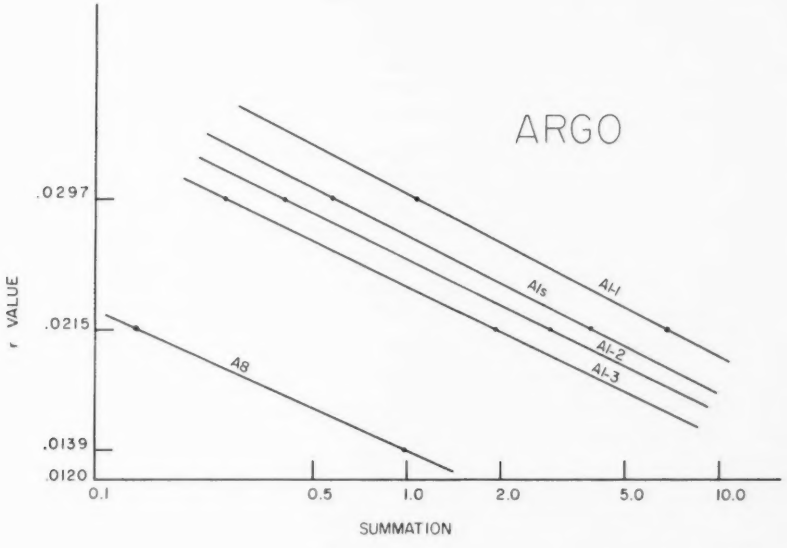

FIGURE 2. Graph showing the summation $\Sigma e^{-rx} l_x m_x$ for trial values of r for snails from Argo.

This linear relation existing between all points plotted from one set of data resulted from the pattern of oviposition; the peak of egg production, in all snails, came early in reproductive life and was followed by a gradual decline (tables 2 and 3). The major portion of the r value is determined by the eggs laid during the early part of the reproductive period. The number of eggs laid after the peak contributed very little to the value. The slope of the line is a function of mean generation time; as the time decreases, the slope becomes steeper (figs. 1 and 2).

DISCUSSION

As was anticipated, the calculated values for r in *Physa gyrina* are less than those for insects (Birch, 1948, 1953a, 1953b; Evans and Smith, 1952; Leslie and Park, 1949) and greater than the values for rats and voles (Leslie, 1945; Leslie and Ranson, 1940). However, the remarkable feature of this study is the range of r for isolates (S1-1 through S1-6) from Scio stock. These values extend beyond the range of r for those snails reared in groups (S2, S6a, S6b) and it is probable that these values for the isolates do not cover the range of individual variation (fig. 1).

Variation in the amount of water (one or two liters), in which isolated snails from the same egg mass were reared, had no correlation with the results obtained. There appears to be, however, a density factor operating. The mortality in egg stages of field collected snails from Scio ranged from 3 to 32 per cent; the mean was 11 per cent. Mortality in self-fertile eggs (table 5) produced by isolated snails (S1-1 through S1-6) ranged from 26 to 52 per cent with a mean of 40. Egg mortalities of two (S2-43 per cent) and six snails (S6a-33 per cent) reared in two liters of water were well within the range of isolated individuals. Six snails (S6b) reared in one liter of water had a mortality of 9 per cent. This suggests that those reared in two liters produced more self-fertile eggs, perhaps as a result of fewer chance meetings and copulations than did those in one liter. The total number of eggs produced per snail by S6a and S6b was not appreciably different (table 5). The per cent of abnormal post-trochophore stages in S6a was 31; in S6b, 7. For isolated individuals (S1-1 through S1-6) this figure ranged from 19 to 47 per cent with a mean of 34. Mortality in self-fertile eggs is highest in post-trochophore stages. These results again suggest that a higher percentage of the eggs produced by S6a were selfed. The morphology and duration of these various life history stages are discussed in a previous paper (DeWitt, 1954a). Since no data for field collected snails from Argo are available, such a comparison cannot be made.

With the exception of S6b, the intrinsic rate of increase for both the Argo and Scio laboratory populations appears to be linearly related to a density factor. Egg production as related to number of individuals reared per container was discussed in another paper (DeWitt, 1954b) before the applicability of the parameter r was called to my attention. The intrinsic rate of increase is higher for S1s and A1s (mean data of isolates) in both populations and decreases as the number of individuals per container is increased.

The intrinsic rate of increase for the two laboratory reared populations (Argo and Scio) are markedly different (table 5). However, it would have been more surprising to find similar values as the two populations came from quite different habitats. Since r is genetically determined, the optimum value within a certain population is the result of selection. The period of development for members of the Argo population is over twice as great as that for the Scio population (table 4). The mean values for intrinsic rate of

increase (r), mean generation time in days (T), and net reproductive rate (R_o) are given below for both populations.

	r	T	R_o
Scio	0.0457	124	418
Argo	0.0235	246	492

As the number of days between birth and oviposition increases (table 4), the value of r falls closer to zero. The net reproductive rate is of the same order in both populations.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the aid of Dr. Frederick E. Smith in the preparation of this paper. Dr. Marcus Goodall kindly verified the validity of the graphic solution.

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Vol. LXXXVIII, No. 842 The American Naturalist September-October, 1954

RADIATION INDUCED MUTATION RATES IN DROSOPHILA AND MICE*

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On the basis of fifty-four mutations induced by 600 r of X-rays at seven selected loci in a total of 48,007 mice, Russell (1951) calculated the average mutation rate to be 25×10^{-5} per r per locus. He compared this rate in particular to the mutation rate observed by Valencia and Muller (1949) at fifteen X-chromosome loci in Drosophila, where the average rate was at most 4×10^{-5} mutations per r per locus, and to the results of some other Drosophila studies where the rates were in some cases perhaps twice as high as the Valencia-Muller rate. He concluded that the radiation-induced mutation rate appeared to be higher in mice than in fruit flies.

Alexander (1952) reported an average rate of 5.7×10^{-5} mutations per r per locus in some 50,000 flies testing the effects of 3000 r of X-rays on the eight loci of the *res* group of mutations in *D. melanogaster*. She interpreted her data on this group of third chromosome loci as supporting Russell's conclusion. However, Russell included in his fifty-four mutants a group of six which died before being tested for mimetic dominant mutation and a group of twelve which had not been tested at the time of publication, these eighteen cases being judged solely on the basis of the F_1 phenotype and Russell's very large previous experience with mouse mutations. On the other hand, all of Alexander's mutations which were used for rate determination were cases of F_2 proven allelism. A much larger portion of her presumptive F_1 mutations either died before the allelism and dominance test could be completed or else were sterile.

Late in 1952, experiments similar to Alexander's were begun in this laboratory, testing various dosages of X-rays and later fast neutrons (Ives *et al.*, 1954). Oregon-R males, raised at 25° C., were exposed to X-rays at a rate of 200 r per minute (120 KV and 10 ma) and were mated singly to *res* females in vial cultures. The F_1 were raised at 25° C. and were scored for visible mutations of all kinds through the eighteenth day after mating.

All *res*-type F_1 individuals have been backcrossed to *res* to test for allelism and dominance. This has revealed that about half of the *roughoid* type of eye mutations were not *ru* at all but were mimetic dominants, at other loci. An occasional *peach*-type mutation has proved to be an allele of *Plum* instead, but with practice one can usually distinguish the variants of those two phenotypes. Virtually no other mimetic dominants have been found.

*The work reported here was supported in part by a grant from the Rockefeller Foundation.

In most series half or more of the *res*-type F_1 mutants have failed in retests because of sterility or inviability. This was also true in Alexander's experiment. The loss of mutant cases at this point is apparently much higher in Drosophila than in mice. Possibly the difference in amount of X-ray dosage is a factor.

In a few cases in the experiments here at Amherst *res*-type F_1 mutants have shown no inheritability of the mutant phenotype in subsequent generations. In other cases unilateral *res*-type mutant F_1 have sometimes given mutant and non-mutant descendants in the tests with *res*. Both types of cases are explainable as delayed mutations which occurred after development had begun and which were included in part or none of the cells of the germ line. Separation of the germ cell line occurs at the third cell division in Drosophila, while in mice it occurs much later, possibly not before the differentiation of the gonadal field.

TABLE 1

NUMBER OF MUTANT F_1 OBSERVED AFTER 3000 r X-RAYS.

Flies	hairy	thread	scarlet	peach	curled	stripe	sooty	Total
13,301	1	2	6	5	6	13	6	39

Russell found that his classification of mouse mutations was accurate for substantially all cases in the F_1 animals. I have found the same to be true for seven of the eight phenotypes of the *res* group in *Drosophila melanogaster*, excluding *roughoid*-like mutants from consideration. Thus it is possible to make reasonably accurate estimates of mutation rates using the F_1 data alone. Much of the data in the present study with X-rays was from treatment with 3000 r. The F_1 results from those series are presented in table 1. Each mutant recorded there occurred as a single bilateral mutation.

The data of table 1 differ from Alexander's chiefly in the relative frequency of *scarlet*, which constituted more than one third of the F_1 mutations in her data when *roughoid* is excluded.

The average mutation rate in the data of table 1 is 14×10^{-5} per r per locus. Assuming that no mimetic mutations were included in the F_1 classification of the same seven phenotypes in Alexander's study, the average mutation rate was 23×10^{-5} per r per locus in her case. Most of the difference between those two rates is accountable by the difference in *scarlet*. In Russell's mouse study there was also one locus, *spotted*, which mutated frequently, accounting for twenty-five of his fifty-four F_1 mutants.

The comparison between the two forms is further complicated, as pointed out by Russell, by the fact that the recovered mutants occurred in mature sperm in the Drosophila and in immature sperm in the mice. In mice the mature sperm are so affected by the radiation that the zygotes resulting from them are abortive. Therefore one does not know the actual mutation rate at the visible loci of mice in mature sperm, and one cannot say whether

it decreases in the same proportion, comparatively, in earlier sperm stages in mice as it does in Drosophila. There may be a difference in the comparative viability of radiation-induced mutant cells in earlier stages of spermatogenesis in Drosophila and mice just as there appears to be in F_1 mutant individuals in the two forms. Russell believed that the observed mutation rate in Drosophila should be halved for comparison with mice in order to compensate for the difference in the developmental stage at which the mutations appear. I do not feel that we know enough about the whole situation yet to justify that procedure.

Considering all the immediate factors involved, it is clear that only a very general comparison of radiation-induced mutation rates can be made between Drosophila and mice at the present time. It is evident that in both organisms some loci mutate more frequently than others and that an average rate based on only a few loci, including one which accounts for nearly half of the observed mutations, is a statistic of very limited meaning. Under these circumstances the fact that the two mutation rates, considering only F_1 results, are anywhere near alike can best be interpreted as suggesting similar radiation-induced mutation rates in the two forms, flies and mice.

It seems important that, in the future, comparison between Drosophila and mouse mutation rates in experiments similar to those discussed here be based only on (1) F_1 results and (2) autosomal loci. The higher mortality and sterility rates of Drosophila F_1 mutants are effects of the mutations, presumably, and have nothing to do with the mutation rate. Therefore, those peculiar characteristics of the Drosophila mutations should not be allowed to bias the calculated mutation rate. At the same time it is important to attempt the genetic identification of the F_1 mutants in order to determine the accuracy of the observer's classification of the F_1. Bilaterally symmetrical F_1 mutants which appear to be delayed mutations in the retest results should be included in the mutant class because the difference of time of separation of germ cell and somatic cell lines in the two forms will bias the mutation rates differentially if these cases are scored only according to inheritability.

The comparison of mutation rates should be made on autosomal loci because there is some evidence that in Drosophila the mutation rate of X-chromosome genes differs significantly from that of autosomal genes. In earlier work (Ives 1945, 1950) the author found the lethal mutation rate in the second chromosome to be probably four times as high as that in the X-chromosome, making allowance for the size difference of the two chromosomes. This was true in both low and high mutation rate stocks. Alexander's data and those reported here suggest an average rate for the seven third chromosome loci which is at least four times as high as the average rate observed by Valencia and Muller for fifteen X-chromosome loci. This difference between autosomal and X-chromosomal mutation rates in Drosophila is much clearer than that existing at present between autosomal mutation rates induced by X-rays in Drosophila and mice.

SUMMARY

This paper discusses the comparison of radiation-induced mutation rates in Drosophila and mice considering principally the data of Russell, Alexander and the author. It is argued that the comparison should be made on the basis of F_1 results using only autosomal loci, for four reasons: (1) differences in viability and fertility of the presumptive F_1 mutants in the two forms; (2) difference in time of separation of germ and somatic cell lines in the two forms; (3) the lack of knowledge on the comparative decrease in proportion of recovered mutations when testing sperm radiated at different developmental ages in the two forms; and (4) apparent differences in mutation rates of X-chromosomal and autosomal loci in Drosophila. Considering also the extreme non-randomness of the mutation rates of the few autosomal loci so far studied it is concluded that for the present the radiation-induced mutation rate per r per locus appears to be similar in flies and mice.

ACKNOWLEDGEMENT

It is a pleasure to acknowledge the help and criticism of Professor H. H. Plough and Dr. H. T. Yost, Jr., in this work.

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Vol. LXXXVIII, No. 842 The American Naturalist September-October, 1954

MALE INFERTILITY ASSOCIATED WITH LACK OF LIBIDO IN THE RAT[1,2]

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INTRODUCTION

The occurrence of non-breeding or sexually sluggish male rodents and attempts to stimulate sexual activity in such animals by massive doses of androgen have been reported by several investigators (Stone, 1938; Beach, 1940; Riss and Young, 1954). Varying degrees of success have been obtained ranging from no response to vigorous sexual activity. The fertility of males induced to breed by such treatment has not been closely studied. However, Stone (1938) placed four induced-copulators with females and found that each male sired one or more litters.

Replacement therapy experiments with castrated adult male rats have been performed in attempting to establish maintenance levels of androgens (Nelson and Merckel, 1937; Cutuly et al., 1937; Moore and Price, 1938; Beach and Holz-Tucker, 1949). Different levels were found adequate, possibly due to environmental differences between laboratories, genetic strains involved, or both. Another source of discrepancy, as pointed out by Beach and Holz-Tucker (1949), lies in the criteria used when comparing castrated, treated males with intact males or with preoperative performance. From the published results cited, it appears that the average maintenance levels for adult male rats is in the neighborhood of 0.125 mg. of testosterone propionate or 0.5 mg. of testosterone per day.

A strain of rats brought into this laboratory was found by Craig (1952) to have an incidence of non-breeding males approximately twice as great as in inbred lines from stock previously introduced. Contemporaneous performances under the same environmental conditions were used for comparisons.

This paper deals with the effectiveness of androgen administration in initiating and maintaining breeding in low libido males, the relative fertility of natural and induced-breeders and autopsy information bearing on the possible endocrine basis for lack of male libido.

MATERIAL AND METHODS

Rats of a commercial strain were introduced into this laboratory in October 1949 for a study of selection and mating systems. The shipment in-

[1] Paper from the Department of Genetics, Number 502. Published with the approval of the Director of the Agricultural Experiment Station.

[2] Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

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cluded 26 males and 79 females. Each animal was from a different litter and all were born within a three week period so that no relationship closer than half brother-sister was likely. Twenty-six inbred lines were formed by mating full brother-sister progeny of the original stock. These lines were continued by full brother-sister and sire-daughter matings. Outbred animals derived from the same foundation stock were maintained as a control group in measuring the effects of inbreeding.

All animals in the colony shared the same environmental and general management conditions as described by Chapman (1946). The same ration, consisting of a variety of natural foods, was used throughout the experiment.

A preliminary experiment involving four males and the females which had been exposed to them without becoming pregnant was carried out to determine whether the infertility was due to lack of willingness to breed on the part of the males or the females. No inseminations occurred although each male was exposed to at least three different females for several estrous periods. When the same females were placed with known vigorous breeding males at least one female from the testers of each male was bred.

An historical survey was made to determine the incidence of non-breeding males within all strains present in the colony from the time of introduction of the low fertility strain. Each male was classified as a breeder or non-breeder on the basis of the records of two females selected at random from the first group placed with him under the routine management practices of the colony. Palpable pregnancy in either female within a period of eight weeks classified a male as a breeder while lack of a palpable pregnancy in both females for a similar period classified a male as a non-breeder.

Systematic "mating trials" were set up for experimental purposes. An individual mating trial involved the exposure of a single female to a male for an entire estrous period as indicated by the vaginal smear technique of Long and Evans (1922). Smears were taken each morning on females used in mating trials until the diestrous stage or until sperm or a vaginal plug was found. The female was then removed from the male's cage. A different female was used for each mating trial and not more than two females were placed with a male at any one time. Lack of unexpected pregnancies and the absence of pseudopregnancies, above a small number presumably resulting from smearing, indicated that the technique was adequate for determining whether ejaculation had actually occurred.

Non-breeding males were obtained by subjecting males classified as non-breeders by the survey method to a "screening period" during which five mating trials were made. Only males failing to breed all of the five estrous females during these trials were used for experimental purposes. It is of interest to note that of 67 males classified as non-breeders by the historical survey method and then screened during one period, 60 did not breed, i.e., did not ejaculate. All seven males which bred during the screening period were found to be fertile by subsequent litter production of the females bred. The relative accuracy of the historical survey method is indicated by these results.

Testosterone was subcutaneously injected into 27 non-breeding males with dosages ranging from 0.5 to 25.0 mg. per day. In all but two cases treatment was continued for a period of 16 to 21 days. Approximately ten mating trials per male were uniformly spread over the treatment period.

Males induced to breed by testosterone injections were compared with naturally breeding males of the same strain for rate of breeding and fertility. Naturally breeding males were selected on the basis of having earlier sired several litters under routine breeding procedure. Comparisons for these characteristics were within inbred or outbred male groups. Systematic mating trials were used for these comparisons. Rate of breeding of induced-breeders while on treatment was calculated on the basis of the proportion of females bred after the initial mating. Thus the first mating served only as an indicator that the breeding period had begun.

Autopsy data were collected on untreated non-breeders and on natural-breeders. Comparisons were within inbred or outbred groups. Weights were taken of the testes, ventral prostate, seminal vesicles (fluid expressed) and pituitary for each male. Left testes of outbred males were fixed in Zenker's fixative and stained with haematoxylin and eosin. A gonadotrophic bioassay was run on the fresh pituitaries by injecting a suspension of a single macerated gland into two weanling female rats. The data pertaining to organ weights and bioassay ovary weights were analyzed by analysis of variance. When a significant multiple correlation coefficient was found between the character under study and the body weight and age of the individual a multiple covariance analysis was used in order to adjust for body weight and age effects, Snedecor (1946).

RESULTS AND DISCUSSION

Initiation of Breeding by Testosterone Injections. Results obtained by injections of testosterone into non-breeding males are presented in table 1. Dosage levels ranged from 0.5 to 25.0 mg. per day which appear to be one to fifty times the average maintenance levels for adult albino male rats as indicated previously. Both inbred and outbred males were included in the control and treated groups but are not shown separately due to the small number at any one treatment level. It appears that a higher level of success might have been obtained had all groups been continued on injections for a longer period, as two of the eleven males responding did not do so before the 15th day.

None of the control males bred females, indicating the effectiveness of the screening process in eliminating breeders from the experimental group. There is no obvious trend in the proportion of males responding at the various levels of exogenous testosterone above the 0.5 mg. level. The actual level administered thus appears unimportant so long as a minimal threshold level is supplied. Just where the threshold level may lie for this strain is not clear, but it appears to be below the 2.0 mg. per day level. The three animals injected at 0.5 mg. per day are not enough to indicate whether this level was inadequate.

TABLE 1

EFFECTIVENESS OF TESTOSTERONE TREATMENT IN INITIATING BREEDING IN LOW LIBIDO MALES

Testosterone per day, mg.	Period of treatment (days)	Number of males	Number responding	Time to first mating of males responding (days)
0	....	15	0	....
0.5	16	3	0	....
2.0	16,21	6	3	5,10,17
4.0	21	9	4	5,6,12,15
10.0	20	7	3	11,11,13
25.0	4	2	1	11
Totals or averages of all treated		27	11	10.5

Control group includes nine inbred and six outbred; treated group includes sixteen inbred and eleven outbred.

The large individual differences found here in ability of adult sexually inactive male rats to respond to exogenous androgen have also been noted by Stone (1938) and by Beach (1940). Riss and Young (1954), however, were unable to show changes in sexual activity level in any of eight low drive adult male guinea pigs on supra-maintenance levels of testosterone propionate.

Successful stimulation of sexual activity in previously non-breeding males by testosterone in this experiment suggests that either the output of male sex hormone is deficient in such males or that the threshold quantity necessary for libido is particularly high in them. Beach (1942) has proposed that initial failure to mate is not due to androgen insufficiency but rather to a low degree of sensitivity in the nervous mechanisms for sexual arousal, since Stone (1938) found that copulatory activity persisted in induced-copulators for periods up to 68 days after withdrawal of exogenous androgen. Limited data on six males from this experiment do not permit entire agreement with this hypothesis since three males had stopped ejaculating by 15 days and four had stopped within 40 days of the last injection. Partial reconciliation of these diverse results may lie in the criterion used for sexual activity, since ejaculation (used here) is known to stop sooner than copulation as shown by Beach (1944) in castrated adult male rats.

Rate of Breeding and Fertility. Induced and spontaneously breeding males did not differ significantly for rate of breeding (table 2). Thus it appears that testosterone, when effective at all, caused an intensity of libido at least equal to that of natural-breeders.

The normality and fertilizing ability of sperm from induced-breeding males did not differ significantly from that of selected fertile males as measured indirectly by the proportion of bred-females becoming pregnant, proportion of litters produced with living young and litter size at birth (table 2).

Autopsy Information. Comparisons of reproductive organ weights and pituitary weights and gonadotrophic potency bioassays for spontaneously breeding and non-breeding untreated males are shown in table 3. Breeders

TABLE 2

BREEDING RATE AND FERTILITY OF NATURAL AND INDUCED-BREEDER MALES CLASSIFIED AS OUTBRED AND INBRED

Male group	Number of males	Treatment period		Treatment and post-treatment periods					
		Total mating trials	Females bred, %	Total females bred	Females pregnant*		Litters of living young		Litter size
					No.	%	No.	%	
Natural-breeders									
Inbred	6	97	42.3	41	39	95	34	83	6.65
Outbred	4	53	32.1	17	14	82	9	53	6.11
Induced-breeders									
Inbred	4	78	51.3	56	44	79	36	64	6.44
Outbred	7	33	54.5	57	48	94†	41	80	6.61

*Diagnosed by palpation.
†Six of the females bred were discarded immediately, hence the percentages are based on 51 females bred.

were in most cases the same males used as controls for the rate of breeding and fertility comparisons. All animals autopsied appeared to be in good health and breeders and non-breeders could not be distinguished on the basis of external appearance.

TABLE 3

AUTOPSY AND BIOASSAY DATA ON INBRED AND OUTBRED MALES CLASSIFIED AS BREEDERS AND NON-BREEDERS

Kind of males	Number of males	Body wt. gms.	Age mos.	Testes mg.	Prostate mg.	Seminal vesicles* mg.	Pituitary wt. mg.	Pituitary bioassay (Ovary wts.)
Inbred								
Breeders	8	374	10.4	2472	644	450	11.7	50.2
Non-breeders	16	348	9.1	3119	567	354	10.3	41.2
(Difference)		26	1.3	−647‡	77	96†	1.4	9.0
Outbred								
Breeders	4	368	12.8	3449	548	504	11.5	30.1
Non-breeders	12	331	7.8	3240	533	363	9.9	25.4
(Difference)		37	5.0	209	15	141†	1.6†	4.7

*Seminal vesicles empty.
†Difference significant at the 5 per cent level.
‡Difference significant at the 1 per cent level.

Testes punctures or autopsy examination of 67 males classified as non-breeders by the survey method showed sperm to be present in all cases. Testes sections of outbred breeders and non-breeders failed to show any marked differences in sperm production or interstitial tissue.

Testes weights in these comparisons do not shed light on the differences between breeders and non-breeders as these weights were significantly heavier for inbred non-breeders but were lighter (not significantly) for outbred non-breeders.

Weights of the prostate glands and seminal vesicles were taken as indicators of male sex hormone levels. Such comparisons seem justified on the assumption that these glands reflect androgen levels and are not necessarily indicative of degree of sensitivity of the nervous mechanisms associated with sexual arousal. The seminal vesicles were significantly heavier in breeders than in non-breeders while the prostates did not differ significantly. It has been shown by Moore and Gallagher (1930), Moore (1935) and Nelson (1937), among others, that the seminal vesicles are more sensitive to androgen insufficiency than the prostate.

Pituitary weights and gonadotrophic bioassays did not in general differ significantly between breeders and non-breeders.

The autopsy results cited here are taken as evidence that a small but real difference existed in androgen output of breeders and non-breeders of this strain, such that the threshold quantity necessary to insure libido was not present in non-breeders. That the difference was small is indicated by the significant difference found for the seminal vesicles and by the lack of such a difference for the less sensitive prostates. The apparent lack of effect of androgen insufficiency on the pituitaries of non-breeders may

reflect a lack of precision of the measures used. The effectiveness of testosterone in stimulating libido in part of the non-breeders and the tendency for loss of libido after withdrawal of this hormone support this interpretation.

Approximately 80 per cent of the males in the strain studied were found to be non-breeders by the historical survey method. Such an extreme incidence of males lacking libido within a population is of considerable evolutionary interest. If such populations occur under natural environments the effective number of breeding males must be reduced, with the consequence of more rapid reduction in heterozygosity.

SUMMARY

A commercial strain of rats brought into this laboratory was found to have very low fertility. Individual mating trials indicated lack of male libido as the primary cause of infertility.

Testosterone injections at various levels were effective in initiating breeding activity in 11 of 27 males, whereas 15 uninjected controls failed to breed during the same period. Limited data on six induced-breeders indicated a tendency for ejaculations to cease after withdrawal of exogenous testosterone.

Induced-breeding males were found to breed as high a proportion of females as spontaneously breeding males. They were also found to be as fertile when compared on the bases of proportion of females pregnant after being bred, proportion of litters with living young and litter size.

Autopsy information and the response of non-breeding males to androgen and to the withdrawal of exogenous androgen support the interpretation that production of male sex hormone below the threshold quantity necessary to insure libido may be an important cause of male infertility in the rat.

The evolutionary consequence of non-breeding males occurring in natural populations is discussed briefly.

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Vol. LXXXVIII, No. 842 The American Naturalist September-October, 1954

THE RELATION BETWEEN TIME OF DEATH OF LETHAL HOMOZYGOTES AND VIABILITY OF HETEROZYGOTES IN DROSOPHILA[1]

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Extensive studies (Stern and Novitski, 1948; Muller, 1950; Stern et al., 1952; Cordeiro, 1952) have demonstrated that "recessive" lethal factors in Drosophila usually have measurable effects on viability when heterozygous. These effects are large enough to indicate that selection against heterozygotes is a major factor in the elimination of lethals from natural populations.

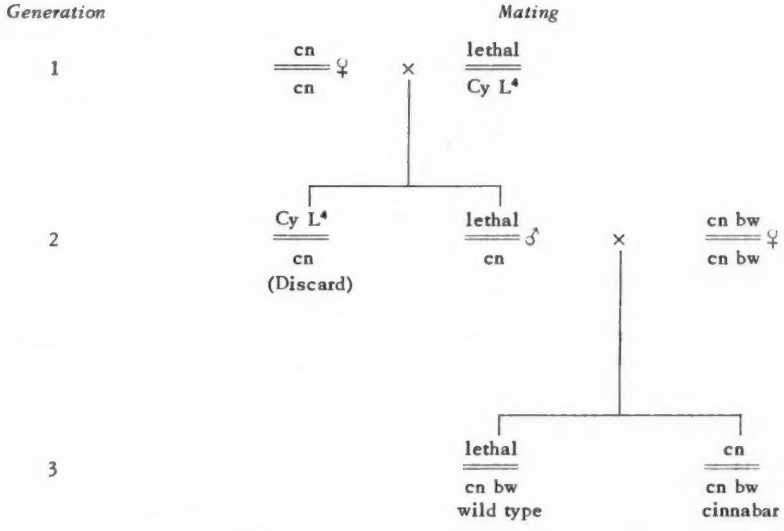
This having been established, there arises the question of the relation between the homozygous and heterozygous effects. Such a comparison is not easily made since ordinarily there is no way of distinguishing between a lethal with a very drastic effect and one barely able to cause death. One approach to the problem is by comparison of heterozygous viability effects of lethals and sub-lethals (Muller, 1950). Another is a comparison of the heterozygous effects of lethals with different times of death in homozygotes, on the assumption that generally those lethals with the most drastic action would result in the earliest death. Seto (1954) has reported the time of action of a series of lethals in *D. melanogaster* and these provided an opportunity for such comparison.

MATERIALS AND METHODS

The lethal-bearing chromosomes were isolated from wild flies collected in the vicinity of Madison, Wisconsin, by the "sifter" technique reported by Muller (1951). Others occurred spontaneously in laboratory stocks or were obtained by X-radiation. All were maintained as stocks balanced with *Cy cn*^{2} *L*^{4} *sp*^{2}, containing the Curly inversions and the dominant markers Curly wing and Lobe eye. The stage of cessation of development of the homozygotes was determined by methods described previously (Seto, 1954).

The crosses made to test the viability effect of the lethal-bearing chromosomes as heterozygotes were as follows. A male heterozygous for the chromosome being tested and for the *Cy L*^{4} chromosome was crossed to *cn* (cinnabar eye) females. The wild type male offspring were then crossed to females homozygous for *cn* and *bw* (brown eye). The *cn bw* combination is white eyed facilitating the detection in the offspring of wrong paternity. The two phenotypic classes of offspring, wild type and *cn*, are expected in equal numbers in the absence of viability differences. The crosses are diagrammed below:

[1]This investigation was supported in part by a grant from the Research Committee with funds provided by the Wisconsin Alumni Research Foundation.



The matings were made in shell vials and kept at about 23° C. The conditions were such that the cultures were moderately crowded. Twenty replicates each containing approximately 75–125 individuals were counted from each mating.

The various lethals were tested for allelism with each other by intercrossing among the balanced lethal stocks. Some instances of apparent allelism (i.e. absence of wild type offspring) were found, and provided an opportunity for a test of the reproducibility of the method. Analysis of variance showed that in no case was there significant heterogeneity in the ratio of *cn* to wild type flies within a set of alleles. Only one member of each set is included in the data presented here.

RESULTS

Relation between time of death in homozygotes and viability in heterozygotes: Table 1 shows the mean ratio of wild type to *cn* flies for 11 egg and egg-larval, 13 larval, and 12 larval-pupal and pupal lethals. The average is about 5 per cent less for the egg and egg-larval lethals than it is for the lethals acting at later stages. In order to determine if this difference was statistically significant, an analysis of variance was performed on the data. This method was particularly suited for the analysis since it can be applied to data involving two or more groups of different sizes. Analysis of variance (table 2) shows that the difference in the ratio in the different stages is significant at the 1 per cent level. However, there is no significant heterogeneity between different lethals acting on the same stage.

These are all lethals obtained from natural populations where there would be some differential elimination of those with the lowest viability

TABLE 1

RELATIVE VIABILITY OF LETHAL HETEROZYGOTES AS MEASURED BY THE RATIO OF WILD TYPE TO CINNABAR FLIES EMERGING. EACH RATIO IS THE MEAN OF 20 REPLICATE EXPERIMENTS

Source of lethal	Lethal stock number	Stage of action	Mean ratio
Wild population	n-15	Egg	0.940
	n-21	Egg	0.952
	n-24	Egg	1.032
	n-26	Egg-larval	1.023
	n-55	Egg-larval	1.028
	n-65	Egg-larval	0.966
	n-69	Egg-larval	0.980
	n-46	Egg-larval	1.026
	n-48	Egg-larval	0.988
	n-31	Egg-larval	0.988
	n-33	Egg-larval	0.990
	mean		.992
Wild population	n-54	Larval	1.065
	n-52	Larval	1.145
	n-3	Larval	1.088
	n-17	Larval	1.076
	n-2	Larval	1.055
	n-14	Larval	0.967
	n-25	Larval	0.907
	n-6	Larval	1.056
	n-56	Larval	1.167
	n-39	Larval	1.120
	n-8	Larval	0.980
	n-16	Larval	1.085
	n-62	Larval	1.084
	mean		1.057
Wild population	n-49	Larval-pupal	0.991
	n-60	Larval-pupal	0.943
	n-34	Larval-pupal	0.928
	n-47	Larval-pupal	1.007
	n-58	Larval-pupal	1.066
	n-28	Larval-pupal	1.062
	n-51	Pupal	1.064
	n-50	Pupal	1.071
	n-4	Pupal	1.200
	n-61	Pupal	1.092
	n-1	Pupal	1.040
	n-32	Pupal	1.092
	mean		1.046
Spontaneous	s-2	Larval	1.069
	s-3	Larval	1.118
	s-9	Larval	1.180
	s-13	Larval	1.035
	s-18	Larval	1.097
	s-28	Larval	1.035
	s-49	Larval	1.007
	mean		1.063

TABLE 1 (*continued*)

Source of lethal	Lethal stock number	Stage of action	Mean ratio
	x-5	Larval	1.012
	x-6	Larval	1.146
	x-7	Larval	1.006
X-rayed	x-27	Larval	0.991
	x-43	Larval	1.068
	x-47	Larval	1.000
	x-63	Larval	0.962
	mean		1.026

as heterozygotes. Thus this study probably underestimates the differences in heterozygote viability that would be obtained if the lethal mutants had been sampled at the time of their occurrence rather than at a later period after some differential elimination of those with greatest heterozygous effects had taken place.

Effect of origin of lethal on heterozygous viability: In addition to those isolated from natural populations, lethals were also obtained as spontaneous occurrences or by X-radiation in a laboratory strain (Mass 38). It has been shown (Rizki, 1952, Seto, 1954) that in general X-ray induced lethals cause cessation of development at an earlier stage than those of spontaneous occurrence, whether the latter are isolated from natural populations or laboratory stocks. For this reason and because, as shown in the last section, the early lethals have a greater heterozygote effect, the X-ray group would have a greater heterozygote effect than the other two groups.

However, one could ask further if, among those lethals acting on the same stage, there was any difference in those of X-ray, spontaneous, and wild population origin. The only stage for which there were large enough numbers in all three groups for comparison was the larval group. The results are shown in table 3.

The data show a difference significant at the 5 per cent level. Thus the chromosomes containing an X-ray induced lethal were associated with a greater reduction of heterozygote viability than those carrying a lethal of spontaneous origin. Since there is no information of the exact time of death within the larval stage, the difference may possibly be due to nothing more than that the X-ray induced lethal homozygotes die earlier in the larval stage.

DISCUSSION

It might be argued that the greater effect on heterozygotes of chromosomes which caused cessation of development at an early stage in homozygotes is due to the occurrence of multiple mutants in some of the chromosomes from natural populations. Such a chromosome would presumably cause development to stop not later than the time at which the earlier of the mutants would have the effect. Thus, multiply-mutant chromosomes would have a higher probability of early death than singly mutant. This

TABLE 2

ANALYSIS OF VARIANCE OF EFFECTS ON HETEROZYGOTE VIABILITY OF LETHALS FROM WILD POPULATIONS WITH DIFFERENT STAGES OF ACTION

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Between stages	2	.711	.355	5.7**
Between stocks within stages	33	2.466	.074	1.2
Within stocks	684	42.935	.063	
Pooled error	717	45.401		

**Significant at the 1 per cent level.

together with cumulative effects of the lethals on heterozygote viability could lead to correlations between earliness of death in homozygotes and reduction of heterozygote viability. It is unlikely that this is a major factor in the results reported here for the following reasons. The total incidence of lethal chromosomes in the natural population sampled was less than 20 per cent. If the number of lethals per chromosome followed a Poisson distribution (probably a conservative assumption, since multiply-mutant chromosomes would be more rapidly eliminated by natural selection), the number of multiply-mutant chromosomes would be less than 2.4 per cent. The proportion of egg and egg-larval mutants which would be multiple, then would not be enough to account for the observed reduction. The stocks were not carried as balanced lethals long enough for there to be any great accumulation of lethals of spontaneous occurrence.

It has long been suggested on several grounds (see Muller, 1950) that recessive factors are generally not completely recessive, and the experiments of Stern et al. and Muller have demonstrated this clearly in the case of lethals. The present study does not offer any additional evidence on this point, since there is no way of making a direct comparison between a lethal-containing chromosome and the same chromosome without the lethal.

However, accepting this conclusion, this study shows a small, but significant correlation between the earliness of the death of the homozygotes and the magnitude of the heterozygous effect. This suggests that the more drastic the homozygous effect (as evidenced by early death) the greater the heterozygous effect.

TABLE 3

ANALYSIS OF VARIANCE OF EFFECTS OF HETEROZYGOTE VIABILITY OF LETHALS ALL ACTING IN THE LARVAL STAGE BUT OF DIFFERENT ORIGIN

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Between origins	2	.383	.193	3.1*
Between stocks within origin	25	1.901	.076	1.2
Within stocks	532	33.334	.063	
Pooled error	557	35.235		

*Significant at the 5 per cent level.

SUMMARY

Second chromosome "recessive" lethals causing development to stop during the egg and egg-larval stage were found to have about 5 per cent greater effect on reduction of viability of heterozygotes than did those acting during the larval and pupal stages.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. James F. Crow for suggesting the mating scheme, the method of analysis and for general advice throughout the course of the experiment.

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Vol. LXXXVIII, No. 842 The American Naturalist September–October, 1954

ABERRATION FREQUENCIES IN IRRADIATED POPULATIONS*

Paget's observations on salivary chromosomes from irradiated Drosophila populations,[1] reported in the last March–April issue of The American Naturalist, reveal higher frequencies of inversions than of translocations. If the relative rates at which inversions and translocations arise in the irradiated populations were known, the difference in observed frequencies could, as Paget suggested, be used to estimate an average difference in the selection pressure against inversion and translocation heterozygotes. The assumption was made that these aberrations arise at about equal rates.

Whereas this assumption has often been found correct for aberrations induced by irradiation of sperm, there are good reasons for supposing that it does not hold for populations in which all stages of gametogenesis in both sexes are exposed to radiation. Glass[2] has found translocations to be much less frequent than inversions after X irradiation of Drosophila females. In Drosophila males, stage differences in absolute sensitivity have been established for dominant[3] and recessive[4] lethals, and it would not be surprising if the relative yields of different aberrations induced at earlier stages were found to be different from those in mature sperm. Translocations from X-rayed Sciara females were found to be extremely rare relative to inversions.[5,6,7] Bozeman and Metz[6] observed many inversions, deletions, and duplications from eggs X-rayed in metaphase or anaphase I. Eggs in prophase gave fewer total aberrations, and relatively more duplications. Irradiation of Sciara sperm[7,8] produced translocations and inversions with essentially equal frequency, as in Drosophila.

These instances may well reflect a rather general situation; as has often been suggested, the spatial arrangement of the chromosomes at some stages may be such that the opportunities for the formation of translocations and inversions are very unequal. In any case, relative or absolute aberration frequencies determined for one cell type and stage are useless as a guide to the rates of origin of the various aberrations in a population. In the irradiated Drosophila populations it may be a permissible simplification at present to assume that equal number of translocations and inversions originate in the males. Added to these are some inversions, but only a negligible number of translocations from the females. It is evident, then, that the relative frequencies of different aberrations are not solely the result of differential selection, but are also determined in part by a higher rate of origin of inversions than of translocations. Therefore the average selection pressure against the inversions must in fact be somewhat greater than Paget estimated on the basis of equal rates of origin.

*Manuscript prepared under Contract W-7405-eng-26 for the Atomic Energy Commission.

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A SEX DIFFERENCE IN THE COAT COLOR CHANGE OF A SPECIFIC GUINEA PIG GENOTYPE*

The purpose of this study was to ascertain the relationship of ovarian estrogens and testicular androgens to a sex difference in the change of coat color with age of a "dingy" brown guinea pig genotype (*E-bbC-PP*) first reported by Wright[4]. As the number of animals available turned out to be rather small and was insufficient for the direct determination of the effects of injections of androgens, the results reported here should be taken only as an indication of the relationships of these hormones to the coat color change rather than as a definitive statement of these relationships. They are reported here as it is impossible at the present time to continue and complete these experiments.

The "dingy" character of the coat color is due to a variable number and distribution of dark tipped very pale brown hairs throughout the basic dark brown coat of these guinea pigs. This condition is somewhat similar to one described as "whitish" by Ibsen and Goertzen[1].

MATERIALS AND METHODS

Males and females of the dingy brown genotype *E-bbC-PP* were castrated or ovariectomized respectively within one week after birth.

Between one and three months of age the coat colors of the rumps of all animals, both normal and sterilized, were measured with a Photovolt photoelectric reflection meter, Model 610, by a method described elsewhere[2] using amber, green, and blue filters. The hair on the rumps of the animals was then plucked manually.

Those castrated or ovariectomized animals which were placed in the group to be injected with estrogen received one rat unit of estradiol benzoate daily for a period of thirty days after their rumps had been plucked. This dosage of estrogen was just sufficient to keep the vaginal closure membrane of ovariectomized female guinea pigs open more or less indefinitely[3] and was assumed to be near the maximal estrogen level in the blood during estrus.

At the end of the thirty-day period when the new coat of rump hair was more or less fully grown, the coat colors of the rumps of all animals were again measured with the reflection meter.

The animals were kept in the guinea pig house under the same conditions and were fed the same diet as the rest of Professor Sewall Wright's guinea pig colony.

Genotypes of the animals were determined from their pedigrees and the presence of dinginess.

RESULTS AND DISCUSSION

The large standard errors of most of the classes reflect the extraordinary variability of the changes in the dingy phenotype. There is, however, a

*This investigation was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

darkening of the uninjected castrated males that is significant at the .001 level (−30.3% ± 4.9%). The uninjected ovariectomized females show an average lightening, but no significance can be attached to this as three of the four animals darkened about 10% while one lightened about 100%. The average for the sixteen animals of these two groups, which lacked sex hormones of either sort, was a darkening of 17.9% ±9.2% (significant only at the .10 level).

Hormone present	Class	No.	Mean age (days)	Mean % change in reflection meter readings	S.E.	Prob.
Androgens	♂♂	14	78.3	+12.3	±10.8	>.05
Estrogens	♀♀	6	51.8	−12.2	± 7.2	>.05
	inj. ♀♀	4	44.0	−28.6	±14.3	>.05
	inj. ♂♂	5	35.2	−31.5	±12.5	>.05
	Combined data	15	44.2	−23.0	± 6.3	<.01
Neither of above	♂♂	12	64.5	−30.3	± 4.9	<.001
	♀♀	4	32.8	+19.2	±28.3	>.05
	Combined data	16	56.6	−17.9	± 9.2	>.05
No androgens	Combined data	31	50.6	−20.4	± 5.6	<.01

Since the normal dingy males change relatively little while the normal dingy females tend to darken greatly with age[4], a test primarily of the effect of the ovarian hormone seemed indicated. The data shown in the table indicate that the three classes in which estrogens are present do indeed darken although none significantly at the .05 level. On combining the data of these three classes, however, there is an average darkening of 23.0% ± 6.3% which is significant at the .01 level.

This average does not differ appreciably from that of the classes without either androgens or estrogens and thus gives no indication of an estrogen effect. If all of the five classes that lack androgens are combined, the average darkening is 20.4% ±5.6%, significant at the .01 level.

If now this is compared with results from the normal males, the difference gives a t value of 2.98 which, with 43 degrees of freedom, is significant at the .01 level. The only apparent differential factor is the presence of androgens in the normal males and its absence in the five other classes.

A second plucking about three to six weeks after the end of the first experimental period produced no marked coat color changes in any class. No hormone injections were made.

A reasonable working hypothesis for future work with this genotype would seem to be that testicular androgens prevent the darkening of the coat color which normally occurs with age in these guinea pigs.

ACKNOWLEDGMENTS

I would like to thank Dr. R. Gaunt of Ciba Pharmaceutical Products, Inc., for the supply of estradiol benzoate (Ovocyllin) and Miss Darhl Fore-

man for discussions of the hormonal aspects of this study. As this investigation was started after a discussion of the problem by Professor Sewall Wright, I would like to express my appreciation to him for his help, interest, and encouragement.

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